UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE ODONTOLOGÍA

TESIS DOCTORAL

Cardiometabolic risk factors and periodontitis: association and preventive and therapeutic implications

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Eduardo Montero Solís

DIRECTORES

David Herrera González
Mariano Sanz Alonso

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Madrid, 2020

A mi familia, con todo mi cariño

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Me gustaría tener unas palabras de agradecimiento para aquellos que me han acompañado durante estos años, y que por tanto, han contribuido directamente o indirectamente a la elaboración de esta tesis doctoral. Me siento muy afortunado de poder contar con tantas personas que me han brindado su apoyo, confianza, cariño y consejo durante todo este tiempo.

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PREFACE

This PhD thesis has been developed thanks to the award of a scholarship for the Training of Research Personnel (FPI) of the Complutense University of Madrid (UCM) of 4 years duration, which included a three-month stay in the Department of Clinical and Translational Research, Forsyth Institute (Cambridge, Massachusetts, United States of America), under the direction of Prof. Thomas E. Van Dyke. This doctoral thesis is based on the following five articles:

Study #1. Montero E, Carasol M, Fernández-Meseguer A, Calvo-Bonacho E, García-Margallo MT, Sanz M, Herrera D. (2019) Prediabetes and diabetes prevalence in the Workers´Oral Health Study. *Clinical Oral Investigations* 23 (12): 4233-4241

Study #2. Montero E, Molina A, Carasol M, Fernández-Meseguer A, Calvo-Bonacho E, García-Margallo MT, Sanz M, Herrera D. (2020) The association between metabolic syndrome and periodontitis in Spain: Results from the WORALTH (Workers´ORAL healTH) Study. *Journal of Clinical Periodontology* 00:1-13

Study #3. Montero E, López M, Vidal H, Martínez M, Virto L, Marrero J, Herrera D, Zapatero A, Sanz M. (2020) Impact of periodontal therapy on systemic markers of inflammation in patients with metabolic syndrome: A randomized clinical trial. *Diabetes, Obesity and Metabolism* 22 (11): 2120-2132. DOI: 10.1111/jcpe.13353

Study #4. Montero E, Herrera D, Sanz M, Dhir S, Van Dyke TE, Sima C. (2019) Development and validation of a predictive model for periodontitis using NHANES 2011-2012 data. *Journal of Clinical Periodontology* 46 (4): 420-429

Study #5. Montero E, Matesanz P, Nobili A, Herrera-Pombo JL, Sanz M, Guerrero A, Bujaldón A, Herrera D, on behalf of the SEPA Research Network of Dental Clinics. (2020) Screening of undiagnosed hyperglycemia in the dental setting: the DiabetRisk study. *Journal of Clinical Periodontology. Accepted for publication.*

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Study #5. Montero E, Matesanz P, Nobili A, Herrera-Pombo JL, Sanz M, Guerrero A, Bujaldón A, Herrera D, on behalf of the SEPA Research Network of Dental Clinics. (2020) Screening of undiagnosed hyperglycemia in the dental setting: the DiabetRisk study. *Journal of Clinical Periodontology. (accepted for publication).*

I. ABSTRACT

Background: Periodontitis has been linked to several systemic diseases, most notably diabetes, for which a clear two-way association has been established. However, whether periodontitis is associated with other metabolic conditions, such as metabolic syndrome (MetS), or with other pre-diabetic hyperglycemic states, such as prediabetes, remains questionable. There is scarce evidence from intervention studies to elucidate if periodontal treatment could reduce the cardiovascular risk in patients with MetS. Furthermore, given the relationship between periodontitis and glycemic control, early detection of both conditions could have a positive impact on their prevention and management.

Objectives: The general objective was to evaluate the association between periodontitis and DM and MetS, and in light of this association, to evaluate the positive global synergistic effects of preventive and/or therapeutic strategies aimed at their early diagnosis or management of these diseases. The specific objectives were: (i) to study the association between periodontitis, hyperglycemia (prediabetes and diabetes mellitus) and MetS in a representative sample of the Spanish employed population (Studies #1 and #2); (ii) to determine whether the treatment of periodontitis in patients with MetS could reduce their cardiometabolic risk (Study #3); to develop and validate a predictive model for moderate-to-severe periodontitis using a combination of cardio-metabolic and socio-demographic variables (Study #4); to evaluate the efficacy of different protocols for the detection of undiagnosed diabetes or prediabetes in a network of dental clinics (Study #5).

Methods and Results.

Studies #1 and #2. WORALTH (Workers' ORAL healTH) Study is a cross-sectional survey, conducted on a representative sample of the Spanish employed population, including 5,154 participants. An oral examination following the World Health Organization (WHO) criteria, evaluated the periodontal status using the Community Periodontal Index (CPI) and Clinical Attachment Levels (CAL). Logistic regression analysis with adjustment for potential confounders was used to evaluate the association between periodontitis and prediabetes, diabetes mellitus and MetS. Prediabetes was not associated with CPI or CAL in fully adjusted multivariate logistic regressions models. Diabetes was significantly

associated with subjects having a CPI=4 after adjustment for potential confounders [odds ratio (OR) = 1.9, 95% confidence interval (CI) 1.1; 3.1]. Participants presenting a CPI=4 were more likely to have MetS than subjects with CPI<4 (OR=1.41; 95% CI [1.10; 1.81]; p<0.001).

Study #3. In a parallel-arm, double-blind, randomized controlled clinical trial, 63 patients with MetS and severe periodontitis were randomly assigned to receive either intensive periodontal treatment (IPT; scaling and root planing plus azithromycin 500 mg every day for 3 days) or minimal periodontal treatment (MPT; supragingival professional mechanical plaque removal plus a placebo). The primary outcome was the impact of the tested interventions on high-sensitivity C-reactive protein (hs-CRP) serum levels at 6 months. Adjusting for baseline hs-CRP, sex, age, smoking status and body mass index, hs-CRP at 6 months was 1.2 mg/L (95% CI [0.4; 2.0]; p=0.004) lower in the IPT group than in the MPT group.

Study #4. A subset of 3017 subjects aged >30 years, with >14 teeth present and having received a periodontal examination in addition to data collected on cardio-metabolic risk measures (smoking habit, body mass index [BMI], blood pressure, total cholesterol and glycated hemoglobin [HbA1c]) were used for model development by multivariable logistic regression. A final predictive model including age, gender, ethnicity, HbA1c and smoking habit as variables had 70.0% sensitivity and 67.6% specificity in detecting moderate-to-severe periodontitis in USA adults.

Study #5. A total of 1143 subjects were included in the study. Participants filled a questionnaire considering diabetes risk factors (FINDRISC) and received a periodontal screening examination. Subjects with a slightly elevated score according to the FINDRISC (≥7), received a point-of-care HbA1c and were eventually referred to their physician for confirmatory diagnosis. Receiver Operating Characteristic (ROC) curves were used to assess the performance of various predictive models with confirmed hyperglycaemia as outcome. From this population, 97 (8.5%) were finally diagnosed of diabetes (n=28; 2.5%) or prediabetes (n=69; 6.0%). When only including the results from the FINDRISC questionnaire, the model reported an area under the curve (AUC) of 0.866 (95% CI [0.833; 0.900]). This model significantly improved when the point-of-care HbA1c was added (AUC= 0.961; 95% CI [0.941; 0.980]; p<0.001).

Conclusions: Severe periodontitis is associated with diabetes mellitus and metabolic syndrome in the Spanish employed population. This association is relevant since periodontal therapy reduces the cardiovascular risk in patients with metabolic syndrome and severe periodontitis. Screening of periodontitis in primary care centers and screening of hyperglycaemia in the dental office seem to be feasible and effective in the identification of undiagnosed individuals.

RESUMEN

Introducción: La periodontitis se ha relacionado con diversas enfermedades sistémicas, entre las que destaca la diabetes mellitus (DM), para la que se ha establecido una clara relación bidireccional. Sin embargo, sigue siendo cuestionable si la periodontitis se asocia con otras afecciones metabólicas, como el síndrome metabólico (MetS), o con otros estados hiperglucémicos, como la prediabetes. Del mismo modo, existe escasa evidencia derivada de estudios de intervención que establezca si el tratamiento periodontal podría reducir el riesgo cardiovascular en pacientes con MetS. Además, dada la relación entre periodontitis y control glucémico, la detección precoz de ambas afecciones podría tener un impacto positivo en su prevención y manejo.

Objetivos: El objetivo general fue evaluar la asociación entre periodontitis y DM y MetS, y a la luz de esta asociación, evaluar los efectos sinérgicos globales positivos de las estrategias preventivas y/o terapéuticas dirigidas al diagnóstico precoz o al tratamiento de estas enfermedades. Los objetivos específicos fueron: (i) estudiar la asociación entre periodontitis, hiperglucemia (prediabetes y diabetes mellitus) y MetS en una muestra representativa de la población laboral española (Estudios # 1 y # 2); (ii) determinar si el tratamiento de la periodontitis en pacientes con MetS podría reducir su riesgo cardiometabólico (Estudio # 3); desarrollar y validar un modelo predictivo para periodontitis moderada o avanzada usando una combinación de variables cardiometabólicas y sociodemográficas (Estudio # 4); evaluar la eficacia de diferentes protocolos para la detección de diabetes o prediabetes no diagnosticada en una red de clínicas dentales (Estudio # 5).

Métodos y resultados.

Estudios # 1 y # 2. El estudio WORALTH (salud bucal de los trabajadores) es una encuesta transversal, realizada en una muestra representativa de la población laboral española, que incluyó 5.154 participantes. Un examen oral siguiendo los criterios de la Organización Mundial de la Salud (OMS) evaluó el estado periodontal utilizando el Índice Periodontal Comunitario (IPC) y los Niveles de Inserción Clínica (NIC). Se utilizó un análisis de regresión logística con ajuste para posibles factores de confusión para evaluar la asociación entre periodontitis y prediabetes, diabetes mellitus y MetS. La prediabetes no se asoció significativamente con el IPC o el NIC en modelos ajustados de regresión

logística. La diabetes se asoció significativamente con aquellos sujetos que tenían un IPC= 4 después del ajuste para posibles factores de confusión [odds ratio (OR) = 1,9, intervalo de confianza (IC) del 95% 1,1; 3.1]. Los participantes que presentaban un CPI = 4 tenían más probabilidades de tener MetS que los sujetos con CPI <4 (OR = 1,41; IC del 95% [1,10; 1,81]; p <0,001).

Estudio # 3. En un ensayo clínico controlado aleatorizado a doble ciego con brazos paralelos, 63 pacientes con MetS y periodontitis avanzada fueron asignados al azar a recibir tratamiento periodontal intensivo (TPI; raspado y alisado radicular junto con azitromicina 500 mg una vez al día durante 3 días) o tratamiento periodontal mínimo (TPM; eliminación de placa mecánica profesional supragingival junto con un placebo). La variable respuesta principal fue el impacto de las intervenciones en los niveles séricos de proteína C reactiva de alta sensibilidad (hs-CRP) a los 6 meses. Ajustando para la PCR-hs basal, el sexo, la edad, el tabaquismo y el índice de masa corporal (IMC), la PCR-hs a los 6 meses fue 1,2 mg / L (IC del 95% [0,4; 2,0]; p = 0,004) más baja en el grupo de TPI que en el grupo TPM.

Estudio # 4. Un subconjunto de 3017 sujetos mayores de 30 años, con> 14 dientes presentes y que recibieron un examen periodontal completo además de contar con datos recopilados sobre los principales factores de riesgo cardio-metabólico [hábito tabáquico, IMC, presión arterial, colesterol total y hemoglobina glucosilada (HbA1c)] se utilizaron para el desarrollo de un modelo predictivo mediante regresión logística multivariable. El modelo predictivo final que incluyó la edad, el sexo, la etnia, la HbA1c y el hábito de fumar como variables, tuvo una sensibilidad del 70,0% y una especificidad del 67,6% para detectar periodontitis moderada o avanzada en adultos de EE. UU.

Estudio # 5. Se incluyeron un total de 1143 sujetos que rellenaron un cuestionario (FINDRISC) considerando los principales factores de riesgo para la DM y recibieron un examen periodontal. Los sujetos con una puntuación ligeramente elevada según el cuestionario FINDRISC (≥7), recibieron una determinación de HbA1c con un dispositivo portátil, y en caso necesario, fueron remitidos a su médico para un diagnóstico de confirmación de DM o prediabetes. Se utilizó el área bajo la curva ROC (del inglés “Receiver Operating Characteristic”) para evaluar el rendimiento de varios modelos predictivos, usando como variable de respuesta el diagnóstico de confirmación de hiperglucemia. De esta población, 97 sujetos (8,5%) fueron finalmente diagnosticados

de DM (n = 28; 2,5%) o prediabetes (n = 69; 6,0%). Cuando solo se incluyeron los resultados del cuestionario FINDRISC, el modelo presentó un área bajo la curva (AUC) de 0,866 (IC del 95% [0,833; 0,900]). Este modelo mejoró significativamente cuando se añadió la determinación de la HbA1c mediante un dispositivo portátil (AUC = 0,961; IC del 95% [0,941; 0,980]; p <0,001).

Conclusiones: La periodontitis avanzada se encuentra asociada significativamente al padecimiento de DM y MetS en la población laboral española. Esta asociación es relevante ya que la terapia periodontal reduce el riesgo cardiovascular en pacientes con MetS y periodontitis avanzada. El cribado de la periodontitis en los centros de atención primaria y el cribado de la hiperglucemia en el consultorio odontológico parecen ser viables y eficaces en la identificación de individuos no diagnosticados anteriormente de estas patologías.

II. INTRODUCTION

Periodontitis is a chronic inflammatory disease associated with oral biofilm dysbiosis and an unresolved inflammation leading to destruction of the tooth supporting structures (Papapanou et al., 2018). Severe periodontitis is estimated to affect 796 million people worldwide (G. B. D. Oral Disorders Collaborators et al., 2020), which implies an enormous public health challenge as periodontitis leads to a significant deterioration of oral health-related quality of life (OHrQL) (Buset et al., 2016; Cunha-Cruz, Hujoel, & Kressin, 2007; Gerritsen, Allen, Witter, Bronkhorst, & Creugers, 2010; Graziani & Tsakos, 2020) and a heavy economic burden on health care systems (Listl, Galloway, Mossey, & Marcenes, 2015; Tonetti, Jepsen, Jin, & Otomo-Corgel, 2017).

Current concepts on the aetiology and pathogenesis of periodontitis include not only the activation of immunoinflammatory mechanisms by the subgingival microbiota, but also several behavioural, genetic and systemic factors that may influence the clinical expression of the disease (Kornman, 2008). Among the medical conditions that may impact periodontal health, diabetes mellitus (DM) is the only recognized systemic risk factor formally included in the 2017 classification system of periodontitis (Tonetti, Greenwell, & Kornman, 2018). However, other metabolic disorders, such as obesity or the metabolic syndrome, have been proposed to be associated with the onset and/or progression of periodontitis (Gorman, Kaye, Nunn, & Garcia, 2012; I. Morita et al., 2011; T. Morita et al., 2010), even though more longitudinal studies in different populations are needed before a causal relationship with periodontitis can be clearly established.

In the last 20 years, increasing evidence has shown that periodontitis may also impact systemic health, giving birth to the term *Periodontal Medicine* (Beck, Papapanou, Philips, & Offenbacher, 2019; Genco & Sanz, 2020). Several mechanisms have been demonstrated to be implicated in these associations, including the spread of the infection from the subgingival environment to the circulatory system and from there to distant tissues and organs (bacteraemia) (Reyes, Herrera, Kozarov, Roldan, & Progulske-Fox, 2013), the release of local inflammatory mediators that secondarily influence systemic inflammation (Amar et al., 2003), the activation of adaptive immunity, and

combinations of all these potential mechanisms (Van Dyke & van Winkelhoff, 2013).

To date, periodontitis has been potentially associated with 57 different systemic conditions (Monsarrat et al., 2016), with DM being the one presenting most robust evidence of a bidirectional association (Sanz et al., 2018; Taylor, 2001). Epidemiological evidence has demonstrated that poor glycaemic control correlates with higher prevalence, severity, and progression rate of periodontitis, when compared to normo-glycemic individuals (Borgnakke, Ylostalo, Taylor, & Genco, 2013a; Graziani, Gennai, Solini, & Petrini, 2018). Conversely, periodontitis has been associated with higher incidence of type 2 DM (Demmer, Jacobs, & Desvarieux, 2008; Saito et al., 2004), and significant improvements in glycemic control (measured by the percentage of glycated haemoglobin, HbA1c) have been reported after periodontal therapy (D´Aiuto et al., 2018; S. Engebretson & Kocher, 2013). There is a surprising lack of information in Spain on the prevalence of periodontitis in subjects with normal and abnormal glucose regulation (prediabetes and DM).

Frequently associated with both diabetes and periodontitis are obesity and metabolic syndrome (MetS), consisting on a cluster of metabolic abnormalities, including increased blood pressure, elevated plasma glucose, excess body fat around the waist and abdominal area, and altered cholesterol levels (Alberti et al., 2009). Numerous studies have reported a positive association between body mass index (BMI) ≥ 25 and periodontitis, although the magnitude of this association has varied in different populations (J. Suvan, D'Aiuto, Moles, Petrie, & Donos, 2011; J. E. Suvan et al., 2015). Similarly, in the third National Health and Nutrition Examination Survey (NHANES), individuals ≥ 45 years of age suffering from severe periodontitis were 2.3 times [95% confidence interval (CI): 1.13; 4.47] more likely to present with MetS when compared with unaffected individuals (D'Aiuto et al., 2008). Chronic systemic inflammation, diagnosed by high sensitivity C-reactive protein (hs-CRP) measurements and white blood cell counts, seems to be the main link among these associations (Demmer et al., 2013; Genco & Van Dyke, 2010). This chronic state of systemic inflammation seems to be a common pathophysiological pathway underlying the association between these conditions with a higher risk for cardiovascular diseases (D'Aiuto et al., 2010), in

particular for periodontitis and metabolic syndrome. There is, however, a lack of knowledge on the impact of periodontal therapy in patients affected by MetS and it remains questionable whether periodontal treatment may decrease systemic inflammation in these patients, and therefore, reduce their cardiovascular risk.

1. ABNORMAL GLUCOSE REGULATION (DIABETES MELLITUS AND PREDIABETES)

Diabetes mellitus represents a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2020). More than 400 million people in the world suffer from DM, which corresponds to a global age-standardized prevalence over 8% in the adult population, with more than 3 million people dying annually as a consequence of their hyperglycemia (Organization, 2016). The prevalence is expected to rise up to 10.8% (700 million people) by 2045 (Saeedi et al., 2019).

Long-term elevated blood glucose levels are associated with damage and dysfunction of different organs, including the eyes (retinopathies), kidneys (nephropathies eventually leading to renal failure), nerves (peripheral neuropathies) or other macrovascular and microvascular complications (Forbes & Cooper, 2013). Specifically, patients with DM present an increased incidence of atherosclerotic cardiovascular and cerebrovascular disease (Forbes & Cooper, 2013).

The development of DM, a multifactorial disease, may be the consequence of a combination of different pathogenic processes, including the destruction of pancreatic β-cells, with subsequent insulin deficiency, or abnormalities resulting in resistance to insulin action. These situations usually coexist in the same patients, frequently causing difficulties in determining which is the primary cause of hyperglycemia.

1.1 Classification of Diabetes Mellitus (Figure 1)

The different clinical scenarios considered by the American Diabetes Association (ADA) (American Diabetes Association, 2020) are:

- Type 1 diabetes, resulting from β-cell destruction, usually leading to absolute insulin deficiency. This form accounts for only 5-10% of those with DM, and it was previously named insulin-dependent diabetes or juvenile-onset diabetes. Although it normally presents during childhood or adolescence, it can appear in some adults that retained residual β-cell function sufficient to prevent ketoacidosis for many years. There are even some forms of type 1 diabetes with no evidence of autoimmunity and, therefore, named *idiopathic diabetes*.
- Type 2 diabetes, results from a progressive insulin secretory defect against a background of insulin resistance. This form of diabetes accounts for ≈90-95% of those with DM. It was previously named non-insulin dependent diabetes or adult-onset diabetes, as it encompasses individuals who usually have relative (rather than absolute) insulin deficiency. Most of the patients with type 2 DM are obese or present an increased percentage of abdominal body fat (Bjorntorp, 1991). This form of DM may be present for a long period of time without any clinical symptom, remaining undiagnosed although these patients are at risk of developing diabetes-related complications. Although insulin resistance may improve with weight reduction and/or pharmacological treatment, it is seldomly restored to normal. Family studies have shown a strong heritability (50-60%) for type 2 DM, although the genetics of this form of DM are complex and are not fully defined (Almgren et al., 2011).
- Other specific types of DM include: genetic defects in β-cell or insulin function, pancreatic diseases such as cystic fibrosis, drug-induced DM (e.g., after organ transplantation).
- Gestational diabetes mellitus (GDM). For a long time, GDM was defined as any glucose intolerance initially recognized during pregnancy. However, the definition persisted even if the case did not resolve post-partum and did not exclude the possibility that unrecognized glucose intolerance may have begun before pregnancy. For this reason, the Association of the Diabetes and Pregnancy Study Groups (IADPSG) recommend that high-risk women (e.g. obese) found to have diabetes at the initial prenatal visit, should receive a diagnosis of overt, and not gestational, diabetes (International Association of Diabetes in Pregnancy Study Group Working Group on Outcome et al., 2015). The prevalence

of GDM ranges between 5.8% in European populations, to 12.9% in the Middle East and North African regions (Zhu & Zhang, 2016).

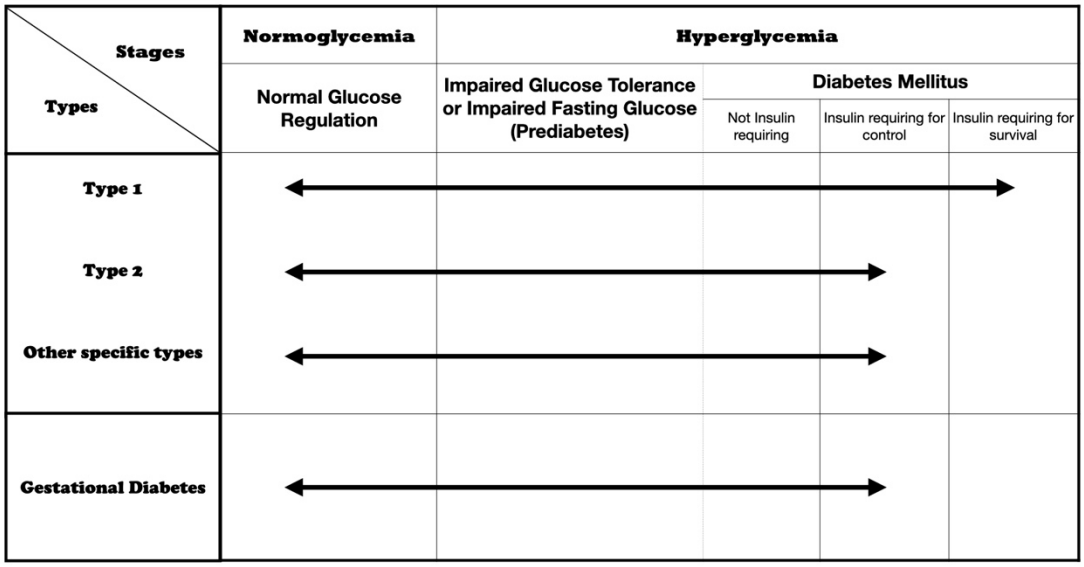

Figure 1. Disorders of glycemia: etiologic types and stages. Adapted from "Diagnosis and classification of diabetes mellitus". American Diabetes Association. Diabetes Care, 2014.

1.2 Diagnostic criteria for diabetes mellitus and categories of increased risk for diabetes

Until the late-1990s, the line between those who had diabetes and those who did not based on blood glucose levels, was set arbitrarily (West, 1975). It was not until the first Expert Committee on the Diagnosis and Classification of Diabetes Mellitus that the diagnostic criteria were revised, based upon the probability of developing microvascular complications (retinopathy in that case). On the basis of three published studies, the glycemic levels, below which there was little retinopathy, and those levels from which prevalence of retinopathy seemed to increase linearly were defined (World Health Organization, 1999). These analyses led to the following long-lasting diagnostic criteria (American Diabetes Association, 2000):

- Two-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test (OGTT). The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
- Fasting Plasma Glucose (FPG) ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours.

In 2013, the ADA introduced a significant change in the diagnosis of diabetes by proposing a hemoglobin A1c (HbA1c) level ≥ 6.5% [following venipuncture and laboratory analyses by a certified National Glycohemoglobin Standardization Program (NGSP) high-performance liquid chromatography (HLPC)] as a criterion for the diagnosis of DM (American Diabetes, 2013). HbA1c is a widely used biomarker to evaluate management of glycemic control, as it reflects the average blood glucose levels over a 2- to 3- month period (Sherwani, Khan, Ekhzaimy, Masood, & Sakharkar, 2016). Apart from the advantage of being already familiar to clinicians as a marker of glycemic control through an extended period of time, the use of HbA1c does not require for fasting, making it more convenient in certain circumstances. However, the cost to evaluate this parameter is higher, and its analyses may be limited in some regions of the developing world and in certain hemoglobinopathies (e.g. anemias from hemolysis or iron deficiency) or congenital ethnic variants may affect the results (Sherwani et al., 2016).

Additionally, patients presenting with severe hyperglycemia symptoms/crisis could be diagnosed by a casual plasma glucose ≥200 mg/dL (11.1 mmol/L).

It is important to understand that there is no 100% concordance between FPG, OGTT and HbA1c as diagnostic criteria for DM, with controversy regarding which biomarker presents a higher sensitivity (Carson, Reynolds, Fonseca, & Muntner, 2010; Ho-Pham, Nguyen, Tran, & Nguyen, 2017). Furthermore, it is also possible that these biomarkers identify different groups of diabetic subjects and physiological processes. Particularly, it seems that HA1c is not sufficiently sensitive to identify those subjects with early diabetes/prediabetes (Cowie et al., 2010; Fajans, Herman, & Oral, 2011).

Since 2003, a group of people have been recognized that do not meet the diagnostic criteria for DM but present blood glucose levels higher than normal (Genuth et al.,

2003). This condition is named prediabetes, and it is defined either as impaired fasting glucose (IFG: FPG levels 100 mg/dL to 125 mg/dL) or impaired glucose tolerance (IGT: OGTT values of 140 mg/dL to 199 mg/dL). The corresponding levels to identify subjects with prediabetes by means of HbA1c are 5.7%-6.4%. IFG mainly represents hepatic insulin sensitivity, while IGT primarily reflects pancreatic β-cell function (Faerch, Borch-Johnsen, Holst, & Vaag, 2009). The Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) is a model to score insulin resistance, while the Homeostatic Model Assessment-β (HOMA-β) scores β-cell function (Matthews et al., 1985).

It has been demonstrated that having prediabetes is a risk factor for developing DM, with a 5-year cumulative incidence ranging from 12% to 25%, which is 3- to 8-fold higher than the incidence for the whole population (Sato et al., 2009; Shimazaki, Kadowaki, Ohyama, Ohe, & Kubota, 2007). Furthermore, prediabetes states have been independently associated to cardiovascular, renal and neurological complications (Fonseca, 2009; Shaye, Amir, Shlomo, & Yechezkel, 2012; Tabak, Herder, Rathmann, Brunner, & Kivimaki, 2012). As long as prediabetes is a reversible condition, individuals with prediabetes should be informed about their increased risk for DM and advised about preventive strategies such as diet modifications or physical exercise. Furthermore, early identification and intervention have been successful in delaying and/or preventing the progression to DM (Diabetes Prevention Program Research Group, 2015).

1.3 Pathogenesis and complications of Diabetes Mellitus

As a consequence of elevated levels of blood glucose, a series of biochemical events occur, including:

i. the increased transformation to sorbitol by the enzyme aldose reductase;

ii. an increased production of diacylglycerol, leading to the activation of protein kinase C;

iii. the formation of non-enzymatic glycation and oxidation products, including HbA1c and the advanced glycation end-products (AGEs).

The first two mechanisms have been proposed to be linked to the development of retinopathies, neuropathies and nephropathies, while the formation of AGEs may alter normal cell function of different cell types, including endothelial cells, mononuclear phagocytes, smooth muscle cells or fibroblasts (Lalla, Lamster, Drury, Fu, & Schmidt, 2000).

Endothelial cell and smooth muscle cells interactions with AGEs may result in the development of a series of events leading to vascular lesions in DM patients (Wautier et al., 1996), while the activation of the receptor for AGEs (RAGE) in inflammatory cells has been linked to the pathogenesis of atherosclerosis and altered responses to infection (Ross, 1999). Lastly, the AGE-RAGE interaction in fibroblasts is an important contributor to the impaired formation and remodeling of the connective tissues observed in DM patients (Ramamurthy & Golub, 1983). All these covert changes may, with time, contribute to the micro- and macrovascular complications of DM. The microvascular complications include nephropathy, retinopathy and neuropathy, while the macrovascular complications comprise cardiovascular diseases resulting into coronary diseases and stroke.

1.3.1 Nephropathy

Diabetes nephropathy is clinically characterized by the development of proteinuria (including albumin as the major component) and, if blood glucose is not properly controlled, subsequent reduced glomerular filtration rate. It is frequently associated with hypertension and it is a major risk factor for macrovascular complications (Matsushita et al., 2010).

1.3.2 Retinopathy

Diabetes retinopathy is the leading cause of blindness in adults. It comprises a series of lesions, including alterations in vascular permeability, microaneurysms and excessive formation of new blood vessels (neovascularization). Initially, changes in the permeability of the blood vessels lead to the accumulation of fluid within the retina, and in late stages, neovascularization and formation of maculae lead to visual impairment and eventually, in retinal detachment (Frank, 2004).

1.3.3 Neuropathy

Diabetic neuropathy affects both the somatic and autonomic peripheral nervous system. The involvement of the somatic nervous system leads to fiber deterioration and progressive loss of sensory perception, while the influence on the autonomic nervous system hampers the ability to maintain vascular tone and an optimum blood flow to the extremities and different organs such as the heart, the brain or the entire gastrointestinal tract (Selvarajah et al., 2006; Wessels et al., 2006). Nowadays, there is no approved therapy for the treatment of diabetic neuropathy.

1.4 Prevention of Diabetes Mellitus

Different approaches have been proposed to prevent the incidence of type 2 DM. Strategically, these initiatives may be separated into those aimed to slow down the progression of chronic hyperglycemia among patients with pre-existing IFG or IGT, and those with the objective of early detection of those subjects at risk of developing diabetes, or those who remain undiagnosed.

The Diabetes Prevention Program (DPP) trial was a landmark study demonstrating that lifestyle interventions, mostly focused on weight loss through diet and physical exercise, are more effective than Metformin to reduce the risk of incident diabetes (Knowler et al., 2002; Perreault et al., 2012). The results of this study, together with its replications in primary care settings, have established lifestyle changes as the cornerstone of diabetes prevention and management (Ackermann, 2013; DeJoy, Padilla, Wilson, Vandenberg, & Davis, 2013).

At the same time, and since up to 37% of the newly diagnosed cases of type 2 DM have already, at least, one diabetes complication (e.g. retinopathy), early diagnosis has become an essential target (Kohner et al., 1998). However, massive screening, measuring either fasting or postprandial plasma glucose is costly and probably inefficient due to the relatively low prevalence of undiagnosed diabetes in the general population. Therefore, selective screening of high-risk subjects identified through self-reported questionnaires, family background and anthropometric measures (e.g. BMI, waist circumference, etc.) has emerged as a cost-effective approach (Lindstrom &

Tuomilehto, 2003). Using this methodology, it has been postulated that the dental practice may offer a valuable opportunity to identify subjects at risk of suffering previously undiagnosed hyperglycemia.

1.5 Management of Diabetes Mellitus

The general management of diabetes patients includes four relevant aspects, described below (Nyenwe, Jerkins, Umpierrez, & Kitabchi, 2011):

1.5.1 *Education*

Patients with abnormal glucose regulation (diabetes or prediabetes) should receive information regarding the disease process, diet and physical exercise advice, instructions in proper blood glucose monitoring and medication intake, and knowledge of acute and chronic complications (Nyenwe et al., 2011).

1.5.2 *Medical nutrition therapy and exercise advice*

Nutrition is a crucial component of a healthy lifestyle. Particularly, in DM patients it has been shown that dietary measures aiming for weight loss through reduction of saturated fats and provision of fiber, whole grains, fruits and vegetables, led to significant improvements in glycemic control and cardiovascular risk factors (i.e. blood pressure and lipid profiles) (Look et al., 2007). Sedentary lifestyle has been identified as a risk factor for DM. A systematic review with meta-analyses published almost 20 years ago reported that a structured moderate exercise program (50 minutes-three times a week) resulted in a 0.7% reduction in HbA1c levels within 8 weeks (Boule, Haddad, Kenny, Wells, & Sigal, 2001).

1.5.3 *Monitoring of glycemic control*

Landmark clinical trials such as the United Kingdom (UK) Prospective Diabetes Study have demonstrated that self-monitoring blood glucose (SMBG) is effective on the reduction of the incidence of DM-associated complications, showing that it is an important component of DM management (Schnell et al., 2013). The advantages of SMBG include the detection of asymptomatic hypoglycemia and hyperglycemic

excursions, which are risk factors for cardiovascular events (Nyenwe et al., 2011). However, it is controversial whether SMBG is effective in type 2 DM patients not treated with insulin (O'Kane, Bunting, Copeland, Coates, & group, 2008).

1.5.4 Drug therapy for glycemic control

A plethora of hypoglycemic agents, including different insulin preparations, are employed in the clinical management hyperglycemia:

Metformin: is considered the first-line agent in the treatment of type 2 DM and in its prevention from prediabetes. It improves peripheral glucose utilization by increasing insulin receptor tyrosine kinase activity (Goodarzi & Bryer-Ash, 2005). It also reduces hepatic gluconeogenesis. It can reduce HbA1c in a range from 0.8% up to 2.0%. Consequently, it has proven to be effective in the reduction of cardiovascular events and to diminish the risk of progression from prediabetes to DM by 31% (Knowler et al., 2002; UK Prospective Diabetes Study (UKPDS) Group, 1998).

Insulin secretagogues: stimulate insulin secretion by the β-cells through interaction with the sulfonylurea receptor, commonly named sulfonylureas for this reason. They are considered a second line of treatment for DM in most patients, mainly due to their side effects (i.e. hypoglycemia and weight gain) (Holstein, Plaschke, & Egberts, 2001). Glinides (e.g., repaglinide) bind to a different part of the sulfonylurea receptor, making them less potent than sulfonylureas but also with less side effects, making them ideal for the combination with metformin (Gerich, Raskin, Jean-Louis, Purkayastha, & Baron, 2005).

Incretins: These are hormones [glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP)] that modulate secretions from pancreatic islet cells, but they can also be administered subcutaneously. They are rapidly inactivated by the enzyme dipeptidyl peptidase IV and, for this reason, they are commonly administered together with dipeptidyl peptidase IV inhibitors (sitagliptin and saxagliptin) (Drucker & Nauck, 2006).

Insulin therapy: Chronic hyperglycemia progression leads to a vicious cycle that further compromise β-cell capacity to secrete insulin, even after administration of hypoglycemic agents. For this reason, most patients with type 2 DM ultimately require insulin therapy to achieve an adequate glycemic control. Human insulin or insulin analogs can be used with this purpose, with the synthetic compounds presenting faster and long-lasting effects, and producing less hypoglycemia and weight gain (Investigators et al., 2006; J. Klein et al., 2004).

Other drugs: Thiazolidinediones (rosiglitazone and pioglitazone) and α-glucosidase inhibitors (acarbose and miglitol) are also regularly prescribed for their ability to ameliorate postprandial hyperglycemia. However, their regular use is limited, mainly due to the controversial side effects of fluid retention (thiazolidinediones) or gastrointestinal effects (α-glucosidase inhibitors).

1.6 Links between periodontitis and abnormal glucose regulation

Different reviews and consensus documents published in recent years have clearly indicated the bidirectional association between diabetes and periodontitis (Chapple & Genco, 2013; Genco & Borgnakke, 2020; Genco, Graziani, & Hasturk, 2020; Lalla & Papapanou, 2011; Preshaw et al., 2012; Sanz et al., 2018). Numerous studies have shown that DM (both type 1 and type 2) is a risk factor for periodontitis, increasing the risk approximately three times when compared with non-diabetes subjects, particularly in patients with diabetes and poor glycemic control (Ide, Hoshuyama, Wilson, Takahashi, & Higashi, 2011a; Nelson et al., 1990; Seppala, Seppala, & Ainamo, 1993; Taylor, Burt, Becker, Genco, Shlossman, et al., 1998). Furthermore, periodontitis negatively affects glycemic control in patients with diabetes, and contributes to the development of complications (Demmer et al., 2010; Shultis et al., 2007).

1.6.1 Mechanistic links underlying the association between periodontitis and diabetes

As previously explained, immune-dysregulation and systemic inflammation are central to the pathogenesis of type 2 DM. The main biological processes and mechanisms that underpin the association between diabetes and periodontitis and vice versa are:

Microbiota: whether patients with periodontitis and DM have a characteristic and distinctive subgingival microbial profile has been a matter of debate for the last 30 years. While early studies observed a significant effect of chronic hyperglycemia in the composition of the periodontal microbiota (Zambon et al., 1988), subsequent reports failed to find a specific bacterial profile among patients with DM (Novaes, Gonzalez Gutierrez, Grisi, & Novaes, 1997; Thorstensson, Dahlen, & Hugoson, 1995). However, the advent of 16s rRNA sequencing methods have offered the possibility to observe the entire microbiome, identifying significant differences in the prevalence or abundance of certain bacteria at several taxonomic levels, identifying certain genus being more prevalent in patients with healthy gums and diabetes (genus of *Neisseria*), or in subjects with both periodontitis and diabetes (*Tannerella forsythia*), when compared with their non-diabetes counterparts (Casarin et al., 2013; M. Zhou et al., 2013). Furthermore, antibody titers against periodontal pathogens have shown a correlation with HbA1c levels (glycemic control) (Merchant et al., 2014). Even though most of these studies have reported an association between poor glycemic control and the periodontal microbiota, there is a need for longitudinal studies assessing the role of these specific microbial profiles in the causal pathway of diabetes.

Host response: Inflammation is a central feature of both diabetes and periodontal diseases, and inflammatory processes are upregulated in the periodontal tissues in patients with diabetes. An altered immune cell response in DM patients has been observed, particularly in neutrophil function. A hyperinflammatory phenotype, releasing increase levels of cytokines, has been characterized after exposure to lipopolysaccharide (LPS), leading to elevated levels of interleukin (IL)-1β and IL-6, that may be responsible for both the periodontal destruction in DM subjects and the contribution of periodontitis to chronic hyperglycemia (Salvi et al., 1997).

Oxidative stress: Both periodontitis and DM have demonstrated to induce oxidative stress locally and systemically (Allen, Matthews, DJ, Griffiths, & Chapple, 2011; Chapple & Matthews, 2007; Evans, Goldfine, Maddux, & Grodsky, 2003). Presence of hyper-active/reactive neutrophils in periodontitis patients generate increased levels of reactive oxygen species (ROS) in response to the dental biofilm, resulting into collateral

host-tissue damage. Furthermore, reduced antioxidant capacity has been previously reported in periodontitis patients (Chapple, 1997). As long as type 2 DM patients with periodontitis have increased plasma biomarkers of oxidative stress (Allen et al., 2011), it may be the case that ROS production by hyper-active/reactive neutrophils in periodontitis could overcome antioxidant defenses, contributing to β-cell dysfunction.

AGEs/RAGEs axis: The binding of AGEs to different cell types in the periodontium may hamper their normal function by leading to pro-inflammatory and pro-oxidant effects (Vlassara & Uribarri, 2014). As an example, it has been proposed that the fibroblasts of DM-affected individuals may lead to abnormal collagen metabolism by reducing its production in the periodontal tissues, eventually contributing to the initiation and progressive destruction of the periodontal support (Claudino et al., 2007). The potential role of AGEs as a mechanistic link between DM and periodontitis have been proved, as serum levels of AGEs demonstrated to be associated with the severity of periodontitis in type 2 DM patients (Takeda et al., 2006).

Bone homeostasis: Several studies have focused on the impact of the most important determinant for bone resorption in DM, the ratio between the receptor activator of nuclear factor κ B ligand (RANKL)/osteoprotegerin (OPG). The soluble RANKL expression and RANKL/OPG ratio has been reported as higher in patients with poorly controlled diabetes when compared with well controlled subjects (Ribeiro et al., 2011; Santos et al., 2010). This finding may explain the increased alveolar bone loss observed among DM patients.

1.6.2 Chronic hyperglycemia as a risk factor for periodontitis

There is plenty of literature stating that type 1 and type 2 DM patients, as well as women with gestational diabetes, suffer more frequently from periodontitis and present with more severe forms of the disease (Chavarry, Vettore, Sansone, & Sheiham, 2009; Cianciola, Park, Bruck, Mosovich, & Genco, 1982; Novak, Taylor, Dawson, Ferguson, & Novak, 2006; Thorstensson & Hugoson, 1993). The strongest evidence is derived from the longitudinal studies carried out in Pima Indians, where it was proven for the first time that DM patients presented a 2.6 times greater risk to suffer periodontitis than

those with normal glucose levels (Nelson et al., 1990). Lately, this finding has been confirmed in other populations with different geographic and ethnic origins, such as the participants in the Study of Health in Pomerania, in Germany (Demmer, Holtfreter, et al., 2012). A particularly higher risk for progression of periodontitis has been observed in those DM subjects with poor glycemic control (Guzman, Karima, Wang, & Van Dyke, 2003; Taylor, Burt, Becker, Genco, & Shlossman, 1998). Indeed, it is abnormal glucose regulation, and not necessarily the diagnosis of DM, that has been correlated with the severity of periodontitis (Saito et al., 2004).

1.6.3 Impact of periodontitis on diabetes

Systematic reviews, with or without meta-analyses have reported consistently a worst glycemic control (either measured as FPG, HBA1c or through OGTT) in patients without overt diabetes when comparing subjects with periodontitis versus non-periodontitis (Borgnakke, Ylostalo, Taylor, & Genco, 2013b; Graziani et al., 2018). However, it is questionable whether there is a true independent association between periodontitis and prediabetes (either measured as IFG, IGT or HbA1c 5.7%-6.4%), or if this association applies only for specific subgroups (e.g. obese or overweight individuals) or geographical areas (Kowall et al., 2015; Rao Deepika & Saxena, 2013).

The longitudinal studies performed in the Gila River Indian community have been important to determine the effect of periodontitis not just on the deterioration of glycemic control, but also on the incidence of diabetes complications in subjects with overt DM. In these studies, periodontitis was associated with a significantly higher incidence of diabetic nephropathy and future ischemic heart disease (Saremi et al., 2005). Subjects with diabetes and severe periodontitis had 3.2 times the risk of death from a cardiovascular or renal event, when compared with diabetes subjects with no/mild/moderate periodontitis. More recent studies have also observed associations between periodontitis and incident retinopathy or even neuropathic foot ulceration (Abrao, Chagas, & Schmid, 2010; Noma et al., 2004). Therefore, it seems clear that periodontitis contributes to an increased risk of development of diabetes complications (Borgnakke et al., 2013b; Graziani et al., 2018).

It is currently a matter of debate whether periodontitis patients with no manifest DM have a greater risk of developing type 2 DM, than those with a better periodontal status. In other words, if periodontitis is a risk factor for the onset of DM. A series of studies, most of them performed in Asian populations (Japan and Taiwan), have reported that subjects with poor periodontal health may have an increased risk for developing DM (Demmer et al., 2010; Demmer et al., 2008; Ide, Hoshuyama, Wilson, Takahashi, & Higashi, 2011b; I. Morita et al., 2012; Saito et al., 2004) (Chiu et al., 2015; Lin et al., 2014). However, some of these studies are retrospective and/or periodontitis was diagnosed by indirect evidence (partial-mouth examinations, surrogate measures such as tooth loss or simplified index scores), what may overestimate the association between the periodontal disease and incident diabetes. Furthermore, some of them failed to prove any association after adjusting for potential confounders. Therefore, there is a need for more studies with accurate measures for both periodontal status and DM (i.e. laboratory data) to determine if periodontitis may have an effect on hyperglycemia in otherwise healthy individuals.

1.6.4 Effect of periodontal treatment on diabetes outcomes

Periodontal treatment reduces inflammation and reduces circulating cytokines among individuals with diabetes (Artese et al., 2015) and may thus decrease hyperglycaemia in these subjects. Numerous randomized clinical trials (RCTs) have been performed since 1998 evaluating the effect of periodontal therapy (consisting in most of the cases of scaling and root planning (SRP) with or without systemic antimicrobials) on HbA1c levels in DM patients. In parallel to the publication of these trials, a series of systematic reviews, with or without meta-analyses, have also been published.

In 2012, a systematic review and meta-analyses on the effect of periodontal treatment on HbA1c levels was presented and discussed at the joint workshop of the European Federation of Periodontology (EFP) and American Academy of Periodontology on “Periodontitis and systemic diseases”. That systematic review reported a mean HbA1c reduction of 0.36% after periodontal therapy (S. Engebretson & Kocher, 2013). However, it was highlighted by the authors that the conclusions should be cautiously considered, due to the small sample size and the high risk of bias of some of the included

studies. To overcome these limitations, a multicenter RCT (Diabetes and Periodontal Therapy Trial, DPTT) was performed in five academic medical centers in the United States of America (USA), including 514 subjects (S. P. Engebretson et al., 2013). However, the DPTT was unable to demonstrate any beneficial effect of periodontal therapy for the reduction of HbA1c levels in patients with DM, and created a great controversy among the scientific community, mainly due to important deficiencies associated with the study design and implementation that could hamper the interpretation of the results (Borgnakke et al., 2014; Chapple, Borgnakke, & Genco, 2014; Merchant, 2014; Vergnes, 2014). First, it seems clear that in order to observe any clinically meaningful improvement in the glycemic control, periodontal treatment would need to reach a minimum standard of care as pre-requisite. Unfortunately, bleeding on probing (BOP) and plaque indices were 41.6% and 72.1% in the test group at the 6 months examination. Secondly, it was difficult to expect any reduction on HbA1c levels as long as they were close to the goal for proper glycemic control at baseline, with approximately 60% of the patients in the study presenting HbA1c levels below 8.0%. A third relevant problem was the mean body mass index (BMI) of the participants, which was approximately 34 kg/m^2, most being obese (BMI≥30 kg/m^2). A recent systematic review concluded that there were significant differences in the metabolic response after periodontal therapy when comparing obese and normal-weight patients (Papageorgiou, Reichert, Jager, & Deschner, 2015), which would have masked the anti-inflammatory effect of periodontal treatment. Thus, it is possible that in the DPTT most of the subjects were resistant to the reduction of systemic inflammation by the periodontal treatment due to the overwhelming influence of obesity.

Several studies have been published since then, and all of them, in agreement with the 9 systematic reviews published during 2013- 2017, have reported reductions in HbA1c at 3-4 months after therapy ranging from 0.27% to 1.03% (Madianos & Koromantzos, 2018). A recent prospective cohort study, including more than 120.000 subjects, with DM and periodontitis treated in the Veteran Administration (VAs) medical centres in the USA, reported that periodontal treatment reduced HbA1c by 0.02% and 0.074% after initial treatment and after an average of 1.7 years of supportive periodontal therapy, respectively (Merchant et al., 2016). Particularly relevant has been a RCT performed in

the United Kingdom and published in *The Lancet Diabetes & Endocrinology* in 2018, including 264 patients (D´Aiuto et al., 2018). Noteworthy, in this investigation, patients in the intensive periodontal therapy group received not only non-surgical subgingival instrumentation but, depending on their needs at the re-evaluation, periodontal surgical therapy to assure optimum subgingival biofilm control. This entire periodontal therapy provided, was considered the standard of specialist care for severe forms of periodontitis, and led in this study to an effective resolution of periodontal inflammation that correlated with an 0.6% reduction in HbA1c levels. Similar observations have been reported in a study using electronic medical records and dental insurance data (n=5103 patients), where those subjects receiving periodontal surgery had 0.25-0.36% lower levels of HbA1c (Spangler et al., 2010).

Therefore, considering all this available evidence, the effects of periodontal treatment on HbA1c points to an improvement of glycaemic control in diabetes patients. The reductions in HbA1c may be clinically significant, as a reduction of 0.2% is associated with a reduction in all-cause mortality of approximately 10% (Khaw et al., 2004), and a reduction of 0.4% is comparable with the one achieved by a secondary-line hypoglycaemic drug (Cavaiola & Pettus, 2000). Furthermore, cost-effectiveness analyses through a life-time have proven that providing periodontal treatment to patients with DM would provide a cost saving, as the reduction in microvascular complications (20.5% for nephropathy, 17.7% for neuropathy and 19.2% for retinopathy) corresponds to a total of net savings close to 6,000 United States dollars (USD) (S. E. Choi, Sima, & Pandya, 2020).

2. METABOLIC SYNDROME

Metabolic syndrome (MetS) is a cluster of metabolic abnormalities that constitute a multiplex risk factor for cardiovascular diseases (Després & Lemieux, 2006). Metabolic syndrome includes abnormal glucose regulation (prediabetes or DM), hypertension, dyslipidaemia and central obesity. The rationale for grouping these conditions is based on the fact that: a) they occur together more frequently than may be expected by chance, and b) together they are associated with an increased risk of cardiovascular disease (Grundy, 2008; Isomaa et al., 2001; Lakka et al., 2002). Although the pathogenic

mechanisms are not completely elucidated, abdominal obesity leading to insulin resistance seems to be the common factor linking the different components (Carr et al., 2004; Reaven, 1988).

Due to the global obesity epidemic, the prevalence of MetS has increased in the last decade, concomitant with an increased risk for diabetes and cardiovascular disease (Grundy, 2008; Zimmet, Alberti, & Shaw, 2001). This increase has been especially pronounced in developing countries. In Spain, the most recent population study reported a prevalence of obesity in 31.2% in adults aged 35-74 years (Martinez-Larrad et al., 2016), which is similar in range to recent European and USA estimates (Aguilar, Bhuket, Torres, Liu, & Wong, 2015; Han & Lean, 2016). The most important determinants in the prevalence of MetS are age and ethnicity, with Hispanics showing the highest rate (Aguilar et al., 2015).

The high prevalence of MetS is relevant from a public health perspective, as people with MetS are three times more likely to have a heart attack or a stroke and have a five times greater risk of developing type 2 DM than people without (Stern, Williams, Gonzalez-Villalpando, Hunt, & Haffner, 2004). Noteworthy, is that as the number of positive components of MetS increase to four or more, the cardiovascular risk increases by six times and the risk of DM ris 35 times (B. E. Klein, Klein, & Lee, 2002). Although there is still some controversy about the practical utility of MetS as a diagnostic or treatment tool, it is considered extremely useful as a research, epidemiological and educational tool (Simmons et al., 2010). Furthermore, when certain specific markers of metabolic alterations (e.g. fasting insulin, apolipoprotein B) are elevated, cardiovascular disease risk increases beyond that reported by traditional absolute risk tools (Lamarche et al., 1998).

2.1 Metabolic syndrome definitions

Several organizations such as the World Health Organization (WHO), the National Cholesterol Education Program – Third Adult Treatment Panel (NCEP ATP III), the International Diabetes Federation (IDF), the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) or the European Group for the Study of Insulin

Resistance (EGIR) have proposed different criteria for the diagnosis of MetS (Alberti et al., 2009). The differences depend upon whether insulin resistance or abdominal obesity are mandatory requirements, or whether the cut-offs for the diagnosis of central obesity were based on waist circumference in different ethnic groups.

Recently, the International Diabetes Federation (IDF) has re-defined MetS, addressing both clinical and research needs, since the existence of multiple definitions becomes confusing and hinders the possibility for direct comparisons among studies (International Diabetes Federation, 2015). According to the new IDF definition, to be diagnosed with MetS, a patient must present central obesity (defined as waist circumference, with specific values according to ethnicity; see Table 1) plus any two of the following three factors: i) lipid abnormality, ii) high blood pressure (BP), iii) hyperglycaemia (see Table 2). Some of the "additional metabolic measurements for research" considered by the IDF definition include antromorphometric measures such as the General body fat distribution (DEXA) or the Central fat distribution (CT/MRI), adipose tissue biomarkers such as leptin or adiponectin, measurements of insulin resistance (Homeostasis Model Assessment -IR), and measurements of pro-inflammatory or prothrombotic states [e.g. CRP, Tumour Necrosis Factor (TNF)-α, IL-6, fibrinogen].

Table 1. Ethnic specific values for waist circumference according to the IDF worldwide definition of MetS (International Diabetes Federation, 2015)

Country/Ethnic group		**Waist circumference**
Europids* In the USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purposes	Male	≥94 cm
	Female	≥80 cm
South Asians Based on a Chinese, Malay and Asian-Indian population	Male	≥90 cm
	Female	≥80 cm
Chinese	Male	≥90 cm
	Female	≥80 cm
Japanese**	Male	≥90 cm

	Female	≥80 cm
Ethnic South and Central Americans	Use South Asian recommendations until more specific data are available	
Sub-Saharan Africans	Use European data until more specific data are available	
Eastern Mediterranean and Middle East (Arab) populations	Use European data until more specific data are available	

* In future epidemiological studies of populations of Europid origin, prevalence should be given using both European and North American cut-points to allow better comparisons.
** Originally different values were proposed for Japanese people, but new data support the use of the values shown above.

Table 2. IDF definition of MetS (International Diabetes Federation, 2015)

Central obesity (defined as waist circumference* with ethnicity specific values) **<u>plus,</u> any two of the following four factors:**	
Raised triglycerides	≥150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality
Reduced HDL cholesterol	<40 mg/dL (1.03 mmol/L) in males <50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality
Raised blood pressure	Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or treatment of previously diagnosed hypertension
Raised fasting plasma glucose	FPG ≥100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 DM If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define the presence of the syndrome

* If body mass index (BMI) is >30 kg/m^2, central obesity can be assumed, and waist circumference does not need to be measured.

HDL, high-density lipoproteins; OGTT, oral glucose tolerance test

2.2 Components of the metabolic syndrome

2.2.1 Central obesity

Obesity is considered the most important "driving force" of the MetS. It is important to note that visceral fat, and not subcutaneous fat, is what increases the risk of metabolic and cardiovascular diseases (Sironi et al., 2012). Imaging techniques, such as magnetic resonance or computed tomography are therefore, the most effective way to assess adiposity (Health, 1998). However, the accessibility and costs of these techniques hamper their regular use, and anthropometric measures are the ones regularly used. As BMI cannot discriminate into lean mass and fat mass, waist circumference has been included as a diagnostic item of metabolic syndrome, although it does not consider height and may underestimate or overestimate obesity in short and tall individuals, respectively (Ashwell, Gunn, & Gibson, 2012). Other novel anthropometric indices have been developed, such as body shape index (ABSI) (Krakauer & Krakauer, 2012), body roundness index (BRI) or body adiposity index (BAI) (Bergman et al., 2011), but they have not been validated or extensively used.

Visceral tissue is not only a compartment designed to store lipids, but also acts as an endocrine organ, releasing pro-inflammatory cytokines (e.g. IL-6, TNF-α). The increase in visceral adipose tissue leads to the release of free fatty acids reaching the liver through the splanchnic circulation and affecting liver metabolism via glucose production and secretion of prothrombotic agents such as plasminogen activator inhibitor 1 (PAI 1) and fibrinogen (Aubert et al., 2003). Furthermore, hypertrophied intra-abdominal adipocytes are resistant to the antilipolytic effect of insulin (Mittelman, Van Citters, Kirkman, & Bergman, 2002). All these mechanisms lead to increased levels of CRP, a biomarker identified as an important predictor of cardiovascular events (Tsimikas, Willerson, & Ridker, 2006).

Interestingly, a subset of obese individuals seems to be protected against metabolic complications and metabolic abnormalities can also be present in non-obese individuals. In fact, approximately 30% of obese subjects in the USA are metabolically healthy (normally considered those with fewer than two cardiometabolic abnormalities), and it remains questionable whether the same approach to treatment based on diet modification and physical exercise can be beneficial, or on the contrary, detrimental, for these subjects.

2.2.2 Insulin resistance and glucose intolerance

Insulin resistance is considered the central mechanism for the development of the MetS. The alterations in insulin normal function include failures in the inhibition of glucose production by the liver and kidney, as well as in the regulation of glucose uptake by different tissues. Initially, when insulin resistance is developed, to ensure proper glycemic control, the body compensates by increasing insulin secretion, and decreasing its clearance. However, with time this mechanism progressively fails, which results in impairment of insulin secretion and, consequently results in alterations in fasting glucose and glucose tolerance.

Insulin action is largely considered as "glucocentric", since this hormone also plays a crucial role in the inhibition of lipolysis in adipose tissue, what leads to an increase in the production of fatty acids that further inhibits the effect of insulin (Jensen, Caruso, Heiling, & Miles, 1989). This mechanism is the one proposed to associate the two main characteristics of MetS: central obesity and insulin resistance.

2.2.3 Dyslipidemia

Dyslipidemia may be a consequence of insulin resistance, since there is an increase in the synthesis of triglycerides in the liver as a consequence of the free fatty acid flux to the liver. Furthermore, in the development of hypertriglyceridemia, a decrease in high density lipoprotein (HDL) cholesterol may occur as a consequence of the decrease in the action of cholesterol ester transfer protein.

2.2.4 Hypertension-High Blood Pressure

Increased blood pressure is another consequence of insulin resistance. This effect has been explained by the vasodilator effect of insulin, which influences sodium resorption in the kidney. In situations of insulin resistance, this vasodilatory effect is lost, while the effect on sodium resorption is preserved, which leads to an increase in blood pressure (Tooke & Hannemann, 2000).

2.5 A bidirectional relationship between periodontitis and metabolic syndrome?

In the recent Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions, obesity and DM were considered as two of the systemic diseases that may affect the periodontal tissues (Albandar, Susin, & Hughes, 2018; Jepsen et al., 2018). Unfortunately, MetS was not evaluated as a distinct entity for its plausible association with periodontitis, even if they present several common inflammatory pathways and several epidemiological studies have reported an association between these two entities.

2.5.1 Mechanistic links underlying the association between periodontitis and metabolic syndrome

The mechanisms that link periodontitis and hyperglycemia, serving as the basis for a two-way relationship between periodontitis and DM, have been previously explained. Besides that, periodontitis has been shown to share a series of pathogenic mechanisms with the rest of MetS components: central obesity, dyslipidemia and hypertension.

Inflammation seems to exert a pivotal role in the association between periodontitis and obesity. Adipose tissue is an important source of several inflammatory mediators produced by adipocytes. Among these mediators, most of them present pro-inflammatory actions (e.g. visfatin, leptin and resistin), while just a few, like adiponectin, present anti-inflammatory characteristics (Adamczak & Wiecek, 2013). Obesity is characterized by a reduction in the levels of adiponectin and, conversely, by an increased production of visfatin, leptin and resistin. These biomarkers of inflammation have been isolated in obese subjects not only in serum, but also in gingival crevicular fluid. Moreover, there is evidence that gingival crevicular fluid levels of adipokines and TNF-α are elevated in obese subjects with periodontitis when compared to periodontitis subjects with normal weight (Zuza et al., 2011). These cytokines present a catabolic action, characterized by the recruitment of immunoinflammatory cells that release proteases resulting into bone resorption (Zimmermann, Bastos, Dias Goncalves, Chambrone, & Duarte, 2013). Therefore, it seems clear that the systemic inflammation induced by obesity may enhance periodontal inflammation in periodontitis subjects, which may result into the destruction of tooth-supporting tissues.

Additionally, periodontitis is a source of pro-inflammatory cytokines and reactive oxygen species (ROS) not only locally, but also systemically (Baltacioglu et al., 2014; Gumus, Nizam, Lappin, & Buduneli, 2014). Periodontitis-associated systemic inflammation can promote insulin resistance, and the resulting hyperinsulinemia may promote obesity by its effects on glucose uptake and fat storage (Demmer, Squillaro, et al., 2012).

However, the association between periodontitis and MetS does not seem to rely solely upon the link with obesity, as periodontitis has proven to be associated with dyslipidemia and hypertension also (Muñoz Aguilera et al., 2020; Nepomuceno et al., 2017). LPS of periodontal pathogens has been demonstrated to be able to increase the lipolytic activity, leading to an up regulation of triglycerides production (Nakarai et al., 2011), and periodontitis has been linked with endothelial dysfunction (Tonetti et al., 2007). However, for these entities the mechanisms are not completely elucidated.

2.5.2 Epidemiological evidence of the association between metabolic syndrome and periodontitis

A recent systematic review with meta-analyses including 20 articles, reported an odds ratio (OR) of 1.71 (95% CI [1.42;2.03]) for the cross-sectional association between periodontitis and metabolic syndrome (Nibali et al., 2013). However, still the association between MetS and periodontitis remains controversial, since the diagnostic criteria used to define the cases of MetS and periodontitis vary between studies, and some studies have only been able to find associations in specific groups, such as women, diabetes patients or in specific age groups (D'Aiuto et al., 2008; Furuta et al., 2013; Sora et al., 2013; Y. K. Tu, D'Aiuto, Lin, Chen, & Chien, 2013), while others have observed no association between MetS and periodontitis (Benguigui et al., 2010; LaMonte et al., 2014; Zuk, Quinonez, Lebenbaum, & Rosella, 2017). Furthermore, just three longitudinal studies on the potential causative relationship between periodontitis and MetS have been published, all of them performed in Japan (I. Morita et al., 2011; T. Morita et al., 2010; Tanaka et al., 2018). Two of them reported significantly higher odds to develop MetS among patients with periodontitis after 4-5 years of follow-up (ORs ranging from 1.4 to 1.6) (T. Morita et al., 2010; Tanaka et al., 2018); while the other longitudinal study

determined that BMI at baseline predicted the 5-year incidence of periodontitis, with a particularly high OR for obese women (OR=3.2)(I. Morita et al., 2011).

2.5.3 Effect of periodontal treatment on outcomes related to metabolic syndrome

Many studies have evaluated the effect of periodontal therapy in obese patients, with conflicting results in regard to its efficacy. While some studies have identified a poorer response to periodontal treatment in obese subjects, others did not observe any difference in outcomes when comparing patients with different BMI categories (Gerber et al., 2016; Papageorgiou et al., 2015). Even if a few trials have shown a reduction in the levels of plasma-reactive oxygen metabolites or adipokines levels in saliva after SRP (Akram et al., 2017; Suresh et al., 2018), further research is needed to determine if periodontal treatment may impact somehow on the metabolic alterations that characterize obesity.

Unlike with obesity, only a few studies have evaluated the impact of periodontal therapy on MetS patients, and all of them have shown a positive effect of periodontal treatment on MetS outcomes [e.g. reductions in CRP and triglycerides, increments of high density lipoprotein (HDL) cholesterol], independently of the treatment modality (supra/subgingival scaling with/without systemic antimicrobials) (Aneesha Acharya, Bhavsar, Jadav, & Parikh, 2010; López et al., 2012; Torumtay et al., 2016). However, there is a high variability in the reported outcomes from these studies, probably due to an unappropriated inclusion of subjects with a high risk for cardiovascular disease, or a lack of proper evaluation of the treatment effect on the etiological infectious burden.

III. JUSTIFICATION

Periodontitis is one of the most prevalent diseases of humankind, with relevant morbidity and associated costs, and with a relevant impact in the quality of life and in overall health, including the association with other chronic non-communicable diseases (NCDs). Among these NCDs, one of the most relevant is the association with DM, due to its bidirectional nature. However, despite the demonstrated association between periodontitis and DM, there are no national reports on the prevalence of periodontitis in subjects with/without normal glucose regulation. Furthermore, the possible association between periodontitis and pre-diabetes or pre-diabetic states has not yet been elucidated.

The potential epidemiological association between other cardiometabolic risk factors (such as obesity or MetS) and periodontitis remains controversial. Similarly, it is unknown which of the individual components of MetS may present the strongest association with periodontitis, or whether this potential association could be independent of the BMI. It would be also interesting to clarify whether non-surgical periodontal therapy can affect the systemic inflammatory status of MetS patients, leading to a reduction in cardiovascular risk in these patients.

Finally, due to the clear relationship between the periodontal condition and overall health, it would be relevant from a public health perspective to reinforce preventive strategies that could be applied in primary care settings for the screening of periodontitis, or in the dental office for the screening of undiagnosed DM or prediabetes, highlighting the collaboration between medical and dental professionals for the management of people at high risk of oral and metabolic conditions, favouring early diagnosis and preventive approaches.

IV. HYPOTHESIS

The general hypothesis of the present work is that there is a bi-directional association between periodontitis and DM and MetS, and in light of this association, preventive and/or therapeutic strategies, aimed at their early diagnosis or management of these diseases, could have positive global synergistic effects, for both individual patients and public health.

Specifically, the following specific hypothesis are proposed:

1. Periodontitis and hyperglycaemia (DM and prediabetes) are significantly associated in the Spanish employed population. Similarly, the presence of a worse periodontal status would be associated with MetS and other indicators of metabolic health, regardless of BMI.

2. Periodontal treatment consisting of scaling and root planning with an adjunctive systemic antimicrobial, would impact the cardiovascular risk profile of patients with MetS and severe periodontitis.

3. A predictive model, combining socio-demographic variables and cardiometabolic risk factors, would be a useful screening tool to identify moderate-to-severe forms of periodontitis.

4. Including a basic periodontal examination as part of a screening protocol for the detection of undiagnosed hyperglycaemia in the dental setting would improve the performance of a patient-reported questionnaire and the use of a point-of-care HbA1c.

V. AIMS

The general objective was to evaluate the bi-directional association between periodontitis and DM and MetS, and in light of this association, to evaluate the positive global synergistic effects of preventive and/or therapeutic strategies aimed at their early diagnosis or management of these diseases.

The specific objectives of the present work were:

1. To study the association between periodontitis and hyperglycemia (diabetes mellitus and prediabetes) in a representative sample of the Spanish employed population ***(Study #1).***

2. To evaluate the association between the periodontal status and the presence of metabolic syndrome (MetS) in a representative sample of the Spanish employed population, and to study the prevalence of periodontitis among metabolically healthy obese (MHO) and metabolically non-healthy non-obese (MNHNO) subjects ***(Study #2).***

3. To determine, in a randomized clinical trial, whether the treatment of periodontitis in patients with MetS can reduce the cardiometabolic risk, measured by means of (i) markers of systemic inflammation and prothrombotic states (hs-CRP, α-1 antitrypsin and fibrinogen levels), (ii) proinflammatory cytokine profiles, and (iii) markers of carbohydrate and lipid metabolism ***(Study #3)***.

4. To develop and to validate a predictive model for moderate-to-severe periodontitis, using a combination of cardio-metabolic and socio-demographic variables from the National Health and Nutrition Examination Survey 2011– 2012 dataset ***(Study #4)***.

5. To evaluate the efficacy of different protocols for the screening of undiagnosed diabetes or prediabetes in a network of dental clinics ***(Study #5).***

VI. MATERIAL AND METHODS. RESULTS

The detailed description of the Material and Methods, as well as the Results of the scientific articles included in the present work have been published in five independent publications with the following references:

Study #1. Montero E, Carasol M, Fernández-Meseguer A, Calvo-Bonacho E, García-Margallo MT, Sanz M, Herrera D. (2019) Prediabetes and diabetes prevalence in the Workers´Oral Health Study. *Clinical Oral Investigations* 23 (12): 4233-4241

Study #2. Montero E, Molina A, Carasol M, Fernández-Meseguer A, Calvo-Bonacho E, García-Margallo MT, Sanz M, Herrera D. (2020) The association between metabolic syndrome and periodontitis in Spain: Results from the WORALTH (Workers´ORAL healTH) Study. *Journal of Clinical Periodontology (accepted for publication)*

Study #3. Montero E, López M, Vidal H, Martínez M, Virto L, Marrero J, Herrera D, Zapatero A, Sanz M. (2020) Impact of periodontal therapy on systemic markers of inflammation in patients with metabolic syndrome: A randomized clinical trial. *Diabetes, Obesity and Metabolism.* DOI: 10.1111/jcpe.13353

Study #4. Montero E, Herrera D, Sanz M, Dhir S, Van Dyke TE, Sima C. (2019) Development and validation of a predictive model for periodontitis using NHANES 2011-2012 data. *Journal of Clinical Periodontology* 46 (4): 420-429

Study #5. Montero E, Matesanz P, Nobili A, Herrera-Pombo JL, Sanz M, Guerrero A, Bujaldón A, Herrera D, on behalf of the SEPA Research Network of Dental Clinics. (2020) Screening of undiagnosed hyperglycemia in the dental setting: the DiabetRisk study. *Journal of Clinical Periodontology (accepted for publication)*

STUDY #1:

Montero E, Carasol M, Fernández-Meseguer A, Calvo-Bonacho E, García-Margallo MT, Sanz M, Herrera D. (2019) Prediabetes and diabetes prevalence in the Workers´Oral Health Study. *Clinical Oral Investigations* 23 (12): 4233-4241

Prediabetes and diabetes prevalence in the Workers´Oral Health Study

Objective: To examine the association between periodontitis, diabetes, and prediabetes, assessed by fasting plasma glucose (FPG).

Materials and methods: Workers' Oral Health Study is a cross-sectional survey conducted on a representative sample of the Spanish employed population including 5154 participants (59.5% men, aged 16–65). Examination of periodontal status assessed Community Periodontal Index (CPI) and clinical attachment levels (CAL). Biochemical determinations included fasting plasma glucose (FPG), triglycerides, and total cholesterol. Logistic regression analysis with adjustment for potential confounders was used to evaluate the association between periodontitis and abnormal glucose regulation.

Results: Ninety-five participants (2.2%) of the study population had diabetes, while 373 (8.8%) presented prediabetes. Prediabetes was not associated with CPI or CAL in fully adjusted multivariate logistic regressions models. Diabetes was significantly associated with subjects having a CPI 4 after adjustment for potential confounders (odds ratio OR = 1.9, 95% confidence interval (CI) 1.1–3.1). This association was stronger in subjects < 45 years (OR = 4.0, 95% CI 1.2–12.7).

Conclusion: Periodontitis was associated with diabetes mellitus, but not with prediabetes, in a representative sample of the Spanish employed population. The association was stronger for younger subjects, which emphasizes the need for early detection of diabetes in younger patients affected by periodontitis, particularly because periodontal therapy may help to improve glycemic control.

Clinical Oral Investigations
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ORIGINAL ARTICLE

Prediabetes and diabetes prevalence in the Workers' Oral Health Study

Eduardo Montero[1] · Miguel Carasol[1] · Ana Fernández-Meseguer[2] · Eva Calvo-Bonacho[2] · María Teresa García-Margallo[3] · Mariano Sanz[1] · David Herrera[1]

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Abstract

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Conclusion Periodontitis was associated with diabetes mellitus, but not with prediabetes, in a representative sample of the Spanish employed population. The association was stronger for younger subjects, which emphasizes the need for early detection of diabetes in younger patients affected by periodontitis, particularly because periodontal therapy may help to improve glycemic control.

Clinical relevance Periodontitis is associated with diabetes mellitus, having at the same time a negative effect on glycemic control. It is important to develop proper early diagnosis strategies for both conditions, particularly in young male adults.

Keywords Periodontal diseases · Periodontitis · Prediabetes · Diabetes · Dental health surveys

Introduction

Prediabetes (either impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or A1c levels of 5.7–6.4%) is a condition currently relevant since there is evidence that it is a strong predictor for future development of diabetes [1, 2].

Data on fasting glucose levels from epidemiologic studies and surveys in 370 countries with approximately 2.7 million participants have reported a clear global trend towards increased glycemic levels since 1980 [3]. The International Diabetes Federation (IDF) in its 2017 Diabetes Atlas has projected that in 2045 the number of people with IGT, between 20 and 79 years, will increase to 587 million, or 8.3% of the adult population [4]. In Spain, according to the Di@bet.es study, almost 30% of the adult population has some carbohydrate disturbance, with an overall prevalence of diabetes mellitus of 13.8%, and with prevalence rates for IFG and IGT of 3.4% and 9.2% respectively [5]. The health implications of this increase are important since prediabetes itself is not only associated with diabetes development, but it has also been associated with higher frequency of cardiovascular, renal, or neurologic complications [2, 6, 7].

✉ Eduardo Montero
eduardomonterosolis@ucm.es

[1] ETEP (Etiology and Therapy of Periodontal Diseases) Research Group, University Complutense of Madrid, Plaza Ramón y Cajal s/n (Ciudad Universitaria), 28040 Madrid, Spain

[2] Ibermutuamur, Madrid, Spain

[3] Cualtis, Madrid, Spain

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Concomitantly, National Health and Nutrition Examination Survey (NHANES) 2009–2012 in the USA have reported an estimated 46% prevalence of periodontitis in adults 30 years or older, which represents 64.7 million subjects affected of this disease in the USA, with 8.9% being severe periodontitis [8]. Similarly, in Europe, it is estimated that around 50% of the adult population suffers from some form of periodontitis, with more than 10% affected by severe forms of the disease [9–11]. This high prevalence of the severe forms of the disease has been regarded as an important public health problem, not only for its oral implications (tooth loss and decreased masticatory function) but also for its effects on the social and quality of life of those affected [12, 13]. Moreover, severe periodontitis has been associated with different chronic systemic inflammatory diseases, such as diabetes. In fact, there is clear epidemiological evidence on the association between periodontitis and diabetes [14, 15] and on the negative effects of periodontitis on glycemic control [16]. Furthermore, some of the biological mechanisms by which periodontitis is a risk factor for the onset of diabetes, its metabolic control, and the advent of complications have been recently elucidated [17].

In spite of the clear association between severe periodontitis as a significant risk factor for the development of diabetes in previously non-diabetic subjects [18], the possible association between periodontitis and prediabetes or prediabetes states has not yet been elucidated. Although several cross-sectional studies have reported a significant association between periodontitis and prediabetes [19–22], others did not demonstrate this significance [23–26]. In the Study of Health in Pomerania (SHIP) [25], a significant association between periodontitis and edentulousness and poorly controlled type 2 diabetes mellitus was reported, but not with prediabetes or well-controlled diabetes.

The aim of this epidemiological investigation was to study the association between periodontitis and prediabetes in a representative sample of the Spanish employed population. This study was part of a wider survey, WORALTH (**W**orkers' Oral Health), studying the oral health status and oral health care needs of the Spanish adult employed population [11].

Material and methods

Study design

WORALTH Study is an oral epidemiological survey using the WHO criteria for oral health surveys [27] that was conducted on a representative sample of the Spanish employed population, from April 2008 to June 2011. The specific epidemiological methodology of this survey was detailed in the previous publication reporting the periodontal data [11]. In brief, workers were examined during their regulated annual health evaluation within the context of a broader epidemiological study of cardiovascular risk assessment comprising a structured interview, physical examination, and laboratory determinations (ICARIA, Ibermutuamur CArdiovascular RIsk Assessment) [28–31].

After applying a proportionate stratified random sampling method according to the geographical area, age, and gender of workers, 5130 subjects were included in the oral health examination, after excluding 47 subjects who refused to attend the oral examinations and 24 fully edentulous subjects (Fig. 1). The sample size of each stratum had been previously defined in relation with the Spanish Labour Force Survey, 2nd quarter [32], and the protocol had been reviewed and approved by Ibermutuamur Ethics Committee.

Socio-demographic and behavioral variables

These variables were obtained from the medical examination (age, gender, smoking status) and the questionnaire of oral health (country of origin, education and income levels, dental visits). Subjects were stratified in different categories according to the different variables:

- Age: under 25 years old, 25–34 years, 35–44 years, 45–54 years, and 55 years or older.
- Smoking habits: never, former (who quit at least 12 months ago), and current smokers.
- Country of origin: from Spain and "other countries".
- Occupation: white-collar (non-manual occupations) and blue-collar (manual occupations)
- Education level: low (primary school), medium (secondary school), or high (university).
- Income level (based on the net income of the family unit): ≤ 1200 euro/month, 1200–3600 euro/month, and > 3600 euro/month.
- Visits to the dentist: regular visits (at least once during the previous year) and irregular

General health conditions

Data from physical examinations included weight, height, waist circumference, and two measures of blood pressure. Body mass index (BMI) was calculated and stratified in three groups: obese (≥ 30 kg/m^2), overweight (25–29 kg/m^2), and normal (< 25 kg/m^2). Waist circumference (WC) was measured while the subject was standing, using the midpoint between the lowest rib and the iliac crest as a reference, and subjects were stratified in Normal (< 94 cm in males and < 80 cm in females), High (≥ 94 cm in males and ≥ 80 cm in females), and Very High (≥ 102 cm in males and ≥ 88 cm in females) [33].

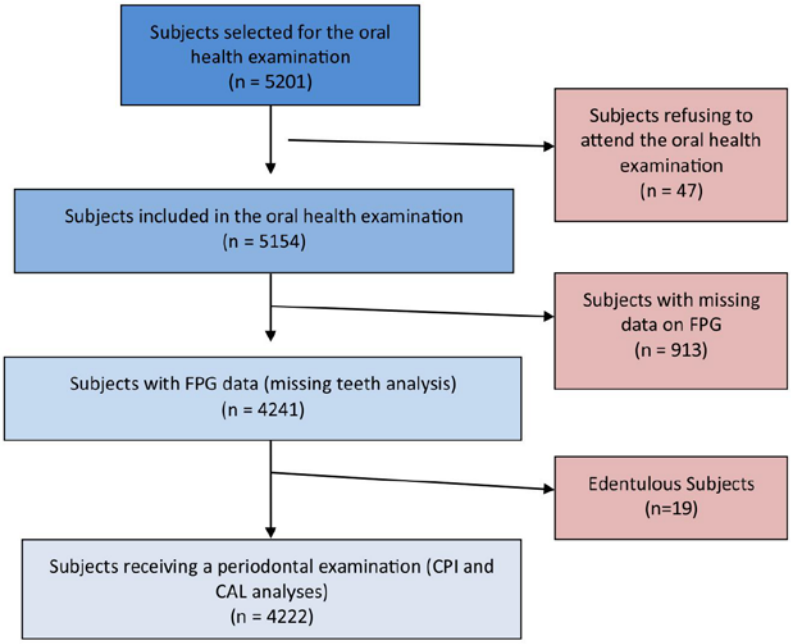

Fig. 1 Flow-chart of the subjects included/excluded in the WORALTH study and in the glycemic control analysis. FPG fasting plasma glucose, CPI Community Periodontal Index, CAL clinical attachment loss

Biochemical analysis

Analyses were carried out in reference laboratories using standard protocols and following Spanish Society of Clinical Biochemistry and Molecular Pathology's (SEQC) quality control recommendations. The variation coefficient for the main serum analyses was within the range accepted by the SEQC [34].

Subjects were advised to fast 12 h prior to the blood analysis. The biochemical determinations included fasting plasma glucose (FPG), triglycerides, total cholesterol, and high-density lipoprotein (HDL)-cholesterol. Low-density lipoprotein (LDL)-cholesterol was also calculated using Friedewald's equation [35].

According to the FPG, subjects were categorized into three groups [36]:

- Normal glucose level: FPG < 100 mg/dL (5.6 mmol/L)
- Prediabetes: FPG ≥ 100 but < 126 mg/dL (7.0 mmol/L)
- Diabetes: (i) FPG ≥ 126 mg/dL, (ii) were taking medication for diabetes, or (iii) they had a prior diagnosis of diabetes

Oral and periodontal examination

Following the WHO criteria [27], periodontal conditions were assessed by the Community Periodontal Index (CPI) and clinical attachment levels (CAL). The ten index teeth were assessed at three buccal sites (mesiobuccal, midbuccal, distobuccal) and three lingual sites (mesiolingual, midlingual, distolingual), and the highest value was recorded at each sextant. Training and calibration sessions were conducted by an experienced WHO epidemiologist, and crossed-examinations with gold standard were carried out to determine the degree of inter-examiner agreement. Calibration data was provided in the previous publication [11].

Data analysis

Participants were excluded from analyses in case of missing data on fasting plasma glucose ($n = 913$). The final analyses included 4241 participants, of which 4222 received a periodontal examination.

Descriptive statistics were calculated by categories of FPG (normal glucose level, prediabetes, and diabetes). Three sets of binomial logistic regression analyses were carried out with the following dependent variables: (i) subjects who had as highest code CPI 4, (ii) subjects with CAL ≥ 6 mm, and (iii) subjects with ≤ 15 missing teeth versus subjects with ≥ 16 missing teeth. The independent variable was glucose regulation (diabetes mellitus, prediabetes, and normal glucose regulation as the reference category). For each of the three sets of logistic regression analyses, three different models are presented as follows: (i) an unadjusted model; (ii) a model adjusted for age and sex; and (iii) a model adjusted for age, sex, occupation, education, smoking, BMI, waist circumference, triglycerides, total cholesterol, systolic/diastolic blood pressure, and frequency of dental attendance.

The analyses were carried out using STATA version 13.1 with SVY package (StataCorp, College Station, TX, USA).

Results

The sample consisted of 60.58% males with a mean age of 38.69 years (standard deviation (SD) 10.89). In Table 1, the characteristics of the participants were described according to the pre-established fasting glucose level categories. Ninety-five participants had diabetes (2.24%) and 373 IFG (8.80%). Unadjusted comparisons showed that age, gender (male), educational level, occupation (white collar/blue collar), smoking habit, BMI, waist circumference, triglycerides, total cholesterol, and systolic/diastolic blood pressure were associated with poorer glucose regulation.

Table 1 Characteristics of participants by categories of glucose regulation ($N = 4241$)

	n	Normal glucose level	Prediabetes	Diabetes	*p* value
n (%)	4241	3773 (89.0%)	373 (8.8%)	95 (2.2%)	
Gender					< 0.001
Male	2569	2200 (85.6%)	281 (10.9%)	88 (3.4%)	
Female	1672	1573 (94.1%)	92 (5.5%)	7 (0.4%)	
Age (years)					< 0.001
< 25	377	359 (95.2%)	18 (4.8%)	0 (0%)	
25–34	1339	1272 (95.0%)	62 (4.6%)	5 (0.4%)	
35–44	1239	1137 (91.8%)	89 (7.2%)	13 (1.1%)	
45–54	862	705 (81.8%)	120 (13.9%)	37 (4.3%)	
≥ 55	424	300 (70.8%)	84 (19.8%)	40 (9.4%)	
Occupation					< 0.001
White collar	2225	2028 (91.2%)	165 (7.5%)	32 (1.4%)	
Blue collar	2016	1745 (86.6%)	208 (10.3%)	63 (3.1%)	
Country of origin					0.065
Spain	3656	3252 (89.0%)	319 (8.7%)	85 (2.3%)	
Others	436	400 (91.7%)	30 (6.9%)	6 (1.4%)	
Education					< 0.001
Primary school	1041	868 (83.4%)	124 (11.9%)	49 (4.7%)	
Secondary school	1705	1543 (90.5%)	130 (7.6%)	32 (1.9%)	
University	1345	1245 (92.6%)	90 (6.7%)	10 (0.7%)	
Net income (monthly)					0.312
< 1200 €	1184	1055 (89.1%)	100 (8.5%)	29 (2.5%)	
1201–3600 €	2138	1919 (89.8%)	179 (8.4%)	40 (1.9%)	
> 3600€	436	396 (90.83%)	32 (7.34%)	8 (1.83%)	
Smoking Status					0.020
Non smoker	2017	1834 (90.9%)	157 (7.8%)	26 (1.3%)	
Former smoker	593	487 (82.1%)	77 (13.0%)	29 (4.9%)	
Smoker	1360	1208 (88.8%)	118 (8.7%)	34 (2.5%)	
BMI (kg/m^2)					< 0.001
Normal	1908	1803 (94.5%)	94 (4.9%)	11 (0.6%)	
Overweight	1617	1407 (87.0%)	168 (10.4%)	42 (2.6%)	
Obese	687	537 (78.2%)	109 (15.9%)	41 (6.0%)	
Waist circumference (cm)					< 0.001
Normal	2270	2102 (92.6%)	143 (6.3%)	25 (1.1%)	
High	996	885 (88.9%)	91 (9.1%)	20 (2.0%)	
Very high	975	786 (80.6%)	139 (14.3%)	50 (5.1%)	
Triglycerides (mg/dl)	4225	100.77 (69.92)	131.87 (124.63)	170.92 (114.87)	< 0.001
Total cholesterol (mg/dl)	4212	194.42 (37.40)	211.67 (38.64)	212.72 (41.49)	< 0.001
Systolic blood pressure (mmHg)	3943	119.31 (15.67)	131.09 (18.95)	139.01 (20.69)	< 0.001
Diastolic blood pressure (mmHg)	3943	74.39 (10.69)	70.63 (11.58)	83.18 (11.18)	< 0.001

BMI, body mass index

In Table 2, the associations between the subject's oral/periodontal condition and glucose control were depicted. Subjects with poorer glycemic control tended to present a greater extent of attachment loss and a higher value of CPI ($p < 0.01$). Also, the number of missing teeth or the frequency of dental attendance was associated with altered glucose metabolism ($p < 0.01$).

In unadjusted logistic regression models (Table 3, model 1), subjects with IFG or diabetes were significantly more likely to have a CAL ≥ 6 mm and a CPI 4. A lower number of teeth (≤ 12 teeth) also significantly correlated with abnormal glucose regulation. When using age-sex-adjusted models (model 2), these significant associations between periodontitis and prediabetes and diabetes expressed diminished odds ratios (ORs). In fact, prediabetes was not significantly associated with periodontal destruction, defined either by CAL or CPI. IFG was only significantly associated with presenting ≥ 16 missing teeth (OR = 1.91; 95% confidence interval (CI) 1.08–3.35). In fully adjusted models (model 3; adjusted for age, sex, occupation, education, smoking, BMI, waist circumference, triglycerides, total cholesterol, systolic/diastolic blood pressure, and frequency of dental attendance), the resulting ORs were further reduced for both prediabetes and diabetes. In fact, the association between diabetes and CAL ≥ 6 mm (OR = 0.95; 95% CI 0.52–1.72) and the presence of a lower number of teeth (OR = 1.57; 95% CI 0.62–3.97) was not significant. Only the association between diabetes and CPI 4 (OR = 1.86; 95% CI 1.13–3.07) remained statistically significant.

After stratifying by sex and age (Table 4) and adjusting for all potential confounders, subjects with CPI 4 were significantly more likely to have diabetes in the < 45 years group (OR = 3.97; 95% CI 1.24–12.68) than in the ≥ 45 years (OR = 1.69; 95% CI 0.99–2.90). In females, the presence of deep periodontal pockets (CPI 4) was not significantly associated with altered glucose levels. In males, the severity of periodontitis (CPI 4) increased in subjects with diabetes (OR = 1.88; 95% CI 1.12–3.16), but significant association was not found for IFG.

Discussion

In this epidemiological survey of the Spanish employed population (WORALTH), diabetes mellitus was significantly associated with severe periodontitis (CPI 4) (OR = 1.86; 95% CI 1.13–3.07). Conversely, the association between prediabetes (as defined by IFG) and periodontitis (assessed by CPI and CAL) was not statistically significant.

Although some studies have reported a significant association between periodontitis and prediabetes [19–22, 24], others did not demonstrate a significant association, such as the study of Health in Pomerania (SHIP) [25] using the

Table 2 Risk distribution for periodontal condition based on fasting glucose levels

	Periodontal condition											
	Maximum CPI			Maximum CAL			Missing teeth				Regular dental visit	
	(*n* = 4222)			(*n* = 4222)			(*n* = 4241)				(*n* = 4054)	
	Codes 0–2	Code 3	Code 4	0–3 mm	4–5 mm	≥ 6 mm	0	1–15	16–19	≥ 20	Yes	No
Normal glucose level[a]	2368 (62.95%)	1053 (27.99%)	341 (9.06%)	3043 (80.89%)	479 (12.73%)	240 (6.38%)	1578 (41.82%)	2141 (56.75%)	23 (0.61%)	31 (0.82%)	1907 (52.62%)	1717 (47.38%)
Prediabetes	253 (58.58%)	108 (29.43%)	44 (11.99%)	253 (68.94%)	108 (19.07%)	44 (11.99%)	103 (27.61%)	252 (67.56%)	8 (2.14%)	10 (2.68%)	164 (47.67%)	180 (52.33%)
Diabetes	30 (32.26%)	33 (35.48%)*	30 (32.26%)*	53 (56.99%)	22 (23.66%)*	18 (19.35%)*	13 (13.68%)	75 (78.95%)	1 (1.05%)	6 (6.32%)*	32 (37.21%)	54 (62.79%)*

CPI, Community Periodontal Index; *CAL*, clinical attachment level

[a] Reference category

*Statistically significant difference when comparing with the reference category ($p < 0.01$)

Table 3 Odds ratios (OR) and 95% confidence intervals (95% CI) for different measures of periodontitis and tooth loss by categories of glucose regulation

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
CPI code 4			
Diabetes mellitus	4.73 (3.02–7.42)	1.93 (1.20–3.09)	1.86 (1.13–3.07)
Prediabetes	1.40 (1.00–1.96)	0.79 (0.56–1.12)	0.78 (0.54–1.13)
Normal glucose regulation	1	1	1
CAL ≥ 6 mm			
Diabetes mellitus	3.53 (2.07–6.01)	1.13 (0.65–1.96)	0.95 (0.52–1.72)
Prediabetes	1.99 (1.41–2.80)	1.03 (0.71–1.47)	0.93 (0.63–1.38)
Normal glucose regulation	1	1	1
Missing teeth (≤ 15 vs ≥ 16)			
Diabetes mellitus	5.15 (2.27–11.67)	2.02 (0.87–4.69)	1.57 (0.62–3.97)
Prediabetes	3.43 (1.99–5.90)	1.91 (1.08–3.35)	1.81 (0.98–3.35)
Normal glucose regulation	1	1	1

OR, odds ratio; *CI*, confidence interval; *CPI*, Community Periodontal Index; *CAL*, clinical attachment level

Model 1: crude ORs, no adjustment

Model 2: adjusted for age and sex

Model 3: adjusted for age, sex, occupation, education, smoking, BMI, waist circumference, triglycerides, total cholesterol, and systolic/diastolic blood pressure

European Workshop in Periodontitis case definition [37] and other measures of periodontitis (mean probing pocket depth (PPD) and percentage of sites with CAL ≥ 4 mm). Also, in agreement with the results from this investigation, Noack et al. [23] in a cohort of 100 patients in Germany did not find significant differences between the periodontal condition of subjects with IGT and normal metabolic status.

Other cross-sectional and nationally representative surveys, such as the National Health and Nutrition Examination Survey (NHANES) in the USA or the KNHANES in South Korea depicted inconclusive results. While data from NHANES III (1988–1994) positively correlated IFG with periodontitis measured by CAL and PPD [20], more recent data derived from NHANES 2009–2010 and NHANES 2009–2012 failed to prove that periodontitis, as defined by the Center for Disease Control/American Academy of Periodontology (CDC/AAP) case definitions [38], was more likely in people with IFG [24, 26]. On the contrary, data from KNHANES 2012–2013 identified IFG as a risk indicator of periodontitis (defined by a CPI ≥ 3), although just in its higher range (111–125 mg/dl) [22]. Recently, a subgroup analysis of a multi-center randomized controlled trial suggested that non-surgical periodontal treatment may reduce HbA1c among people with prediabetes [39]. However, other studies evaluating the effect of scaling and root planing on the glycemic control of patients with prediabetes reported inconsistent results [40, 41].

The present study demonstrated a significant association between diabetes and periodontal condition (CPI 4), which is in accordance with most of the published studies reporting a significantly higher prevalence of severe periodontitis in people with diabetes [42, 43]. Specifically, approximately 30% of people with diabetes had severe forms of periodontitis [26]. Other studies also using CPI have reported similar results, with subjects with diabetes being approximately 1.5-fold more likely to suffer from severe periodontitis, after the adjusted logistic regression analysis [22]. Other studies have evaluated the effect of glycemic control on the odds for having periodontitis, with most of these studies reporting significant associations between periodontitis and diabetes only in people with uncontrolled diabetes [25, 26, 44]. In the present study, it was not possible to evaluate the effect of glycemic control as A1c data was not available, and single FPG measurements are

Table 4 Sex and age-stratified Odds Ratios (ORs) and 95% confidence intervals (95% CI) for subjects with Community Periodontal Index (CPI) code 4 by categories of glucose regulation

	Subjects with CPI = 4 OR (95%CI)	
	Prediabetes	Diabetes
Sex		
Female	1.04 (0.45–2.40)	1.24 (0.13–11.61)
Male	0.74 (0.49–1.11)	1.88 (1.12–3.16)
Age		
< 45 years	0.68 (0.29–1.59)	3.97 (1.24–12.68)
≥ 45 years	0.80 (0.53–1.22)	1.69 (0.99–2.90)

Logistic regressions were adjusted for age, sex, occupation, education, smoking, body mass index (BMI), waist circumference, triglycerides, total cholesterol, and systolic/diastolic blood pressure. Normal Glucose Level served as reference category (OR = 1.00)

not adequate to evaluate the metabolic control in diabetes patients.

The strength of the associations between periodontal condition and diabetes also varied among the published studies. In the present investigation, the reported degree was weak (after adjusting for confounders, there was a significant association for CPI, but not for mean CAL or lower number of teeth). This may be due to the low prevalence of diabetes in this sample (2.24%), lower than the prevalence reported in other population-based studies, such as NHANES 2009–2012 in the USA (12.6%) or SHIP-Trend in Germany (11.8%) [25, 26]. The present study is restricted to the employed population, with a mean age (38.8 years) and range (16–65 years) relatively young, which may account for this low prevalence of diabetes, since this disease prevalence significantly increases with age. Indeed, diabetes prevalence in the entire Spanish population is sixfold higher than the one presented in this sample comprising just a working population.

Another possible explanation for the absence of an association between periodontitis and CAL or lower number of teeth underlies on the biological plausibility of the relationship. Both the direct (bacteremia) and indirect (pro-inflammatory cytokines) mechanisms proposed require the presence of an ulcerated pocket epithelium. While CPI is related with the probing pocket depth component, mean CAL and the number of missing teeth could represent gingival recession or past disease, without any impact on the periodontal inflamed surface area (PISA) [45].

This study has also reported differences by sex and age, with periodontitis being significantly associated with diabetes in men, but not in women. Again, these results may have been influenced by the low number of cases of females with diabetes. Similar findings have been reported in NHANES 2009–2012 [26]. Also, when different phenotypes of periodontitis have been proposed based on gingival tissue transcriptomic data, gender was an important independent risk factor for the extent and severity of periodontitis [46]. In terms of age, this study reported a stronger association between diabetes and CPI 4 in adults < 45 years, which may imply a higher aggressiveness of the chronic inflammation secondary to periodontitis or diabetes.

The present study is not free of limitations. The study design is cross-sectional and, therefore, does not allow for a causal or temporal relationship. The included population was limited to employed subjects and did not include older adults, which may reflect the quite low diabetes prevalence in the study. What is more, a single fasting glucose test was performed, while current standards for the diagnosis of diabetes mellitus advise for repeated testing in absence of unequivocal hyperglycemia [36]. As discussed previously, one limitation of this study could emanate from the partial recording protocol used [11]. CPI is not the gold standard in periodontal epidemiology and has several limitations, although this method is highly sensitive for prevalence estimates in presence of 4–6 mm probing pocket levels (≥ 90%) [47].

The major strength of the study was the large, representative sample of the Spanish employed population since periodontal and FPG data were available from 4222 subjects.

In conclusion, this epidemiological survey has found a significant association between severe periodontitis (CPI 4) and diabetes mellitus, especially in men and younger adults (< 45 years). Conversely, there was no significant association between the periodontal health status and prediabetes (assessed by IFG). The present findings highlight the importance of providing oral health education as well as regular periodontal examinations to patients with diabetes. These recommendations are in agreement with the guidelines proposed in the recent workshop on periodontal diseases and diabetes organized by the International Diabetes Federation (IDF) and the European Federation of Periodontology [48].

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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STUDY #2

Montero E, Molina A, Carasol M, Fernández-Meseguer A, Calvo-Bonacho E, García-Margallo MT, Sanz M, Herrera D. (2020) The association between metabolic syndrome and periodontitis in Spain: Results from the WORALTH (Workers´ORAL healTH) Study. *Journal of Clinical Periodontology (accepted for publication)*

The association between metabolic syndrome and periodontitis in Spain: Results from the WORALTH (Workers´ORAL healTH) Study

Introduction: Evidence of an association between periodontitis and MetS (metabolic syndrome) remains controversial. The objective of this study is to evaluate the association between periodontitis and MetS in a cross-sectional population survey.

Material and Methods: WORALTH (Workers' ORAL healTH) Study is a cross-sectional survey, conducted on a representative sample of the Spanish employed population, including 5,154 participants. An oral examination following the World Health Organization (WHO) criteria, evaluated the periodontal status using the Community Periodontal Index (CPI) and Clinical Attachment Levels (CAL). Logistic regression analysis with adjustment for potential confounders was used to evaluate the association between periodontitis and MetS, and its individual components.

Results: Participants presenting a CPI=4 were more likely to have MetS than subjects with CPI<4 [odds ratio, OR=1.41; 95% confidence interval (CI) 1.10-1.81; p<0.001]. High blood pressure was the component with stronger association with periodontal status (OR=1.94 for CAL≥ 6 mm; 95% CI 1.49-2.53; p<0.001). After stratifying for sex, the association was higher in women (OR=2.20 for CPI=4; 95% CI 1.31-3.62; p<0.001). Non-metabolically healthy subjects, obese or not, presented a worse periodontal condition.

Conclusion: Severe periodontitis (CPI=4) was associated with MetS in a representative sample of the Spanish employed population. This association seems to be independent of body mass index and other potential confounders.

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The association between metabolic syndrome and periodontitis in Spain: Results from the *WORALTH (Workers' ORAL healTH) Study*

Eduardo Montero[1] | Ana Molina[1] | Miguel Carasol[1] | Ana Fernández-Meseguer[2] | Eva Calvo-Bonacho[2] | María Teresa García-Margallo[3] | Mariano Sanz[1] | David Herrera[1]

[1]ETEP (Etiology and Therapy of Periodontal and Peri-implant Diseases) Research Group, University Complutense of Madrid, Spain

[2]Ibermutua, Madrid, Spain

[3]Cualtis, Madrid, Spain

Correspondence
Eduardo Montero, Facultad de Odontología, Universidad Complutense de Madrid, Plaza de Ramón y Cajal s/n, 28040 Madrid, Spain.
Email: eduardomonterosolis@ucm.es

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Abstract

Introduction: Evidence of an association between periodontitis and MetS (metabolic syndrome) remains controversial. The objective of this study is to evaluate the association between periodontitis and MetS in a cross-sectional population survey.

Material and Methods: WORALTH (Workers' ORAL healTH) Study is a cross-sectional survey, conducted on a representative sample of the Spanish employed population, including 5154 participants. An oral examination following the World Health Organization (WHO) criteria evaluated the periodontal status using the Community Periodontal Index (CPI) and Clinical Attachment Levels (CAL). Logistic regression analysis with adjustment for potential confounders was used to evaluate the association between periodontitis and MetS, and its individual components.

Results: Participants presenting a CPI = 4 were more likely to have MetS than subjects with CPI < 4 [odds ratio, OR = 1.41; 95% confidence interval (CI) 1.10–1.81; $p < 0.001$]. High blood pressure was the component with stronger association with periodontal status (OR = 1.94 for CAL ≥6 mm; 95% CI 1.49–2.53; $p < 0.001$). After stratifying for sex, the association was higher in women (OR = 2.20 for CPI = 4; 95% CI 1.31–3.62; $p < 0.001$). Non-metabolically healthy subjects, obese or not, presented a worse periodontal condition.

Conclusion: Severe periodontitis (CPI = 4) was associated with MetS in a representative sample of the Spanish employed population. This association seems to be independent of body mass index and other potential confounders.

KEYWORDS
dental health surveys, high blood pressure, impaired glucose regulation, metabolic syndrome, obesity, periodontitis

1 | INTRODUCTION

Periodontitis is a chronic inflammatory disease of infectious origin, defined by destruction of the tooth supporting tissues, which if not treated, may lead to tooth loss. Results from the Global Burden of Disease (GBD) Study, and other epidemiological data, have reported that more than 50% of adults are affected by mild-to-moderate forms of periodontitis, while the severe forms evidence a global age-standardized prevalence of ≈11%, what results in the sixth most prevalent condition in the world (Kassebaum et al., 2014; Petersen & Ogawa, 2012).

Non-communicable diseases (NCDs) like cardiovascular disease (CVD), diabetes mellitus (DM), cancer or chronic respiratory disease are currently the most prevalent diseases affecting humans, what is leading to a health and socio-economic global crisis (Beaglehole et al., 2011). Also, periodontitis is considered as a NCD (Herrera et al., 2018; Tonetti et al., 2017) and different risk factors associated with periodontitis may be shared by the other NCDs, such as smoking, stress, unhealthy diet, glycaemic control, or genetic and socio-economic determinants (Petersen & Ogawa, 2012; Pihlstrom et al., 2005). In addition to the shared risk factors, periodontitis is associated with a low-grade systemic inflammatory response that increases the risk for atherogenesis and hyperglycaemia (Morita et al., 2010; Paraskevas et al., 2008; Reyes et al., 2013). Different specific mechanisms and pathological pathways have been attributed for this significant association between periodontitis and CVD or DM, either through direct mechanisms (bacteraemia or endotoxaemia, e.g. by periodontal pathogens such as *Porphyromonas gingivalis*), and/or indirectly, through systemic inflammation or molecular mimicry-induced autoimmune damage (de Pablo et al., 2009; Reyes et al., 2013; Schenkein & Loos, 2013; Taylor et al., 2013).

Metabolic syndrome (MetS) consists of a cluster of clinical and biological abnormalities, including insulin resistance, central obesity, dyslipidaemia and hypertension that constitute a risk factor for CVD (Eckel et al., 2005). In fact, subjects affected with MetS have twice the risk of developing CVDs (Benguigui et al., 2010). Several studies have reported an increase in MetS prevalence around the world, with a particularly important rise in developing countries (Cameron et al., 2004; Misra & Khurana, 2008; Prasad et al., 2012). In Spain, the most recent population study reported a prevalence of 31.2% in adults aged 35–74 years (Martinez-Larrad et al., 2016), which is in a similar range compared with recent European estimates (Han & Lean, 2016). Recently, the International Diabetes Federation (IDF) has re-defined the MetS, addressing both clinical and research needs, since the existence of multiple definitions had proven to be confusing and to hinder direct comparisons between studies (International Diabetes Federation, 2015). According to the new IDF definition, to be diagnosed with MetS, a patient must present central obesity (defined as waist circumference, with specific values according to ethnicity) plus any two of the following three factors: (a) lipid abnormality, (b) high blood pressure (BP) and (c) hyperglycaemia.

Clinical Relevance

Scientific rationale for study: There is a need to study whether metabolic syndrome (MetS) and periodontitis are associated and whether this potential association just applies for certain subgroups depending on age, sex or presence of other comorbidities.

Principal findings: A worse periodontal condition is associated with significantly higher odds for suffering MetS, independently of the syndrome definition, being high blood pressure the component with a stronger association with periodontitis.

Practical implications: Periodontitis and MetS are significantly associated. Therefore, regular periodontal checkups should be done in patients with MetS. The potential benefits of periodontal therapy in these patients need to be determined.

Moreover, different phenotypes of MetS have been described depending on the relation between the body size and the metabolic profile (Karelis et al., 2004). "Metabolically healthy obese" (MHO) are subjects that, in spite of having obesity, lack any cardiometabolic abnormality. Also, the opposite ["Metabolically non-healthy non-obese" (MNHNO)] refers to those subjects metabolically abnormal, but with normal weight.

The potential association between periodontitis and MetS has been recently reviewed in a systematic review with meta-analysis, reporting an odds ratio (OR) of 1.71 [95% confidence interval (CI) 1.42–2.03] (Nibali et al., 2013). Additionally, conditions associated with MetS, such as erectile dysfunction (Gorgel et al., 2014), have also been proposed to be associated with periodontitis (Bizzarro & Loos, 2019; Zadik et al., 2009). However, still the association between MetS and periodontitis remains controversial, since the diagnostic criteria used to define the case of MetS and of periodontitis vary between studies, and some studies have only been able to find association in specific groups, such as women, diabetic patients or in specific age groups (D'Aiuto et al., 2008; Furuta et al., 2013; Sora et al., 2013; Tu et al., 2013), while others have observed no association between MetS and periodontitis (Benguigui et al., 2010; LaMonte et al., 2014; Zuk et al., 2017). Hence, there is a need of further research to clarify these inconsistencies and heterogeneous results among distinct groups.

It was, therefore, the objective of the present epidemiological investigation to evaluate a representative sample of the Spanish employed population to study the association between their periodontal status and the presence of MetS. As secondary objective, we also evaluated the prevalence of periodontitis among MHO and MNHNO subjects. This study was part of a wider survey, the WORALTH (Workers' ORAL healTH), studying the oral health

status and oral healthcare needs of the Spanish adult employed population (Carasol et al., 2016).

2 | METHODS

2.1 | Study design

The WORALTH Study was conducted on a representative sample of the employed population in Spain, from April 2008 to June 2011. It is an epidemiological survey using the WHO criteria for Oral Health Surveys (WHO, 1997), with a specific methodology to assess the subject's periodontal condition, which has been reported in a previous publication (Carasol et al., 2016). In brief, workers, within the context of a broader epidemiological study on cardiovascular risk assessment, were examined during their regular annual health evaluation by means of a structured interview, physical examination and laboratory determinations (ICARIA, Ibermutuamur CArdiovascular RIsk Assessment) (Sanchez-Chaparro et al., 2006, 2008, 2011; Valdivielso et al., 2009).

After applying a proportionate stratified random sampling method, depending on geographical area, age and gender, 5201 workers were screened for inclusion and, finally, 5130 subjects were included in the oral health examination, since 47 subjects refused to participate and 24 were fully edentulous. The sample size of each segment had been previously calculated in relation to the Spanish Labour Force Survey, 2nd quarter (Instituto Nacional de Estadística, 2008). The protocol of this investigation was reviewed and approved by Ibermutuamur Ethics Committee. This study conforms with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting cross-sectional studies.

2.2 | Socio-demographic and behavioural variables

These variables were obtained from the medical examination (age, gender, smoking status) and the oral health questionnaire (country of origin, education and income levels, dental visits). Subjects were stratified in different categories for each variable, as follows:

- Age: <25 years old, 25–34 years, 35–44 years, 45–54 years, or ≥55 years.
- Smoking habits: never, former (who quit at least 12 months before) or current smokers.
- Country of origin: Spain or other countries.
- Occupation: white-collar (non-manual occupations) or blue-collar (manual occupations).
- Education level: low (primary school), medium (secondary school) or high (university).
- Income level (based on the net income of the family unit): ≤1200 €/month, 1.200–3.600 €/month and >3.600 €/month.
- Visits to the dentist: regular visits (at least once during the previous year) or irregular attendance.

2.3 | Physical examination

Waist circumference (WC) was measured while the subject was standing, using the midpoint between the lowest rib and the iliac crest as a reference. Additional data from physical examinations included weight, height and two measurements of BP. Body mass index (BMI) was calculated, and subjects were stratified in three groups according to its value: obese (≥30 kg/m^2), overweight (25–29 kg/m^2) and normal (<25 kg/m^2).

2.4 | Biochemical analysis

Serum samples were analysed in reference laboratories using standard protocols, variation coefficients and ranges accepted by the Spanish Society of Clinical Biochemistry and Molecular Pathology's (SEQC) (Sacks et al., 2011). Subjects were advised to fast 12 hours prior to taking the blood sample. The biochemical determinations included fasting plasma glucose (FPG), triglycerides, total cholesterol and high-density lipoprotein (HDL) cholesterol. Low density lipoprotein (LDL) cholesterol was also calculated using Friedewald's equation (Friedewald et al., 1972).

2.5 | Oral and periodontal examination

Following an oral examination using the WHO criteria (WHO, 1997), the periodontal condition was assessed by means of the Community Periodontal Index (CPI) and the registration of clinical attachment levels (CAL). The ten index teeth were assessed at three buccal sites (mesiobuccal, midbuccal, distobuccal) and three lingual sites (mesiolingual, midlingual, distolingual) and the highest value was recorded at each sextant. Training and calibration sessions were conducted by an experienced WHO epidemiologist, and crossed-examinations with gold standard were carried out to determine the degree of inter-examiner agreement. Calibration data were provided in a previous publication (Carasol et al., 2016).

2.6 | Metabolic syndrome definitions

Two different MetS definitions were used in this study: i) the one released by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) (National Cholesterol Education Program Expert Panel on Detection & Treatment of High Blood Cholesterol in, 2002); and ii) the standardized diagnostic criteria for MetS according to the IDF consensus worldwide definition (International Diabetes Federation, 2015).

According to the NCEP-ATP III (National Cholesterol Education Program Expert Panel on Detection & Treatment of High Blood Cholesterol in, 2002), a subject is diagnosed of MetS if he/she has three or more of the following criteria:

- Central obesity (defined as waist circumference ≥102 cm in men and ≥88 cm in women).
- Hypertriglyceridaemia (≥150 mg/dl).
- Low HDL cholesterol (<40 mg/dl in males, <50 mg/dl in females).
- High BP (systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg).
- High fasting glucose (>110 mg/dl).

According to the IDF consensus definition (International Diabetes Federation, 2015), a subject with MetS is defined when presenting central obesity (defined as waist circumference ≥94 cm in males and ≥80 cm in females, in populations of European origin), and any two of the following factors:

- High triglyceride levels (≥150 mg/dl or specific treatment for this lipid abnormality).
- Reduced HDL cholesterol (<40 mg/dl in males, <50 mg/dl in females or specific treatment for this lipid abnormality).
- High BP (systolic BP ≥130 mmHg, or diastolic BP ≥85 mmHg or treatment of previously diagnosed hypertension).
- High fasting plasma glucose (fasting plasma glucose ≥100 mg/dl or previously diagnosed of type 2 diabetes).

2.7 | Body size phenotype definitions

In the present study, we used the criteria proposed by Wildman et al. (Wildman et al., 2008) for defining body size phenotypes. Metabolically abnormal phenotype was defined by the presence of ≥2 cardiometabolic abnormalities (from those ones included in the IDF consensus definition for MetS). The study population was stratified by BMI categories (normal weight and overweight versus obese) and metabolic phenotype, thus generating four body size phenotypes: metabolically healthy non-obese (MHNO), metabolically healthy obese (MHO), metabolically non-healthy non-obese (MNHNO) and metabolically non-healthy obese (MNHO).

2.8 | Data analysis

Data from subjects without enough information for the diagnosis of MetS were excluded from the analyses (n = 777). Hence, the final analyses included 4353 participants. Descriptive statistics were calculated for demographic and clinical characteristics, by diagnosis of MetS, according to the different definitions, using chi-squared tests, for categorical variables, and t tests and analysis of variance, for continuous variables. Similar analyses were performed for the different body size phenotypes. Different sets of binomial logistic regression analyses were carried out using the following dependent variables: (a) subjects who had as highest code CPI = 4, (b) subjects with CAL ≥ 6 mm and (c) subjects with ≤15 missing teeth versus subjects with ≥16 missing teeth. The independent variables were either the different definitions of MetS, their components or the body size phenotype. For each of the sets of logistic regression analyses, two different models were calculated: (a) a crude model and (b) an adjusted model considering all potential confounders. The analyses were carried out using STATA version 13.1 with SVY package (StataCorp).

3 | RESULTS

The sample consisted of 4353 subjects, with 2623 men (60.1%) and 1730 women (39.9%). Demographic and clinical characteristics of the sample by MetS status are shown in Table 1. Overall, prevalence of MetS was 8.5% using NCEP-ATP III criteria and 16.2% using IDF criteria. Subjects with MetS were older, had lower educational level and were smokers or former smokers, when compared to subjects without MetS. In addition, patients with MetS (with both definitions) presented higher BP, more abdominal obesity, lower levels of HDL and higher levels of triglycerides and fasting plasma glucose, which are components of the syndrome definition. A limited proportion of patients were undergoing drug treatment for diabetes (n = 87; 2.0%), high blood pressure (n = 221; 5.1%) or lipid-lowering drugs (n = 288; 6.6%).

Periodontal evaluation and oral health behaviour indicators are shown in Table 2. The percentage of subjects with periodontal pockets (CPI codes 3–4) was significantly higher among patients with MetS, when compared with patients without MetS, independently of the definition (52.0% versus 35.1%, respectively, with IDF criteria, $p < 0.001$; 52.5% versus 36.4%, respectively, with ATP III criteria, $p < 0.001$). The same occurred for the proportion of subjects with attachment loss ≥6 mm (12.9% versus 6.0%, respectively, with IDF criteria, $p < 0.001$; 14.4% versus 6.5%, respectively, with ATP III criteria, $p < 0.001$), or with the number of remaining teeth (subjects diagnosed of MetS presented significantly lower number of teeth; $p < 0.001$). Subjects brushing their teeth at least twice a day were less frequently diagnosed of MetS, independently of the definition, when compared with subjects with MetS (46.0% versus 54.0%, using the IDF criteria, $p < 0.001$; or 45.1% versus 54.9%, respectively, using the ATP III criteria, $p < 0.001$).

Table 3 and S1 describe the characteristics of the participants for each MetS component (based on the IDF definition) according to the CPI or CAL category. Subjects who have as highest code a CPI = 3–4 were significantly associated with more abdominal obesity, hypertriglyceridaemia, high BP and low HDL cholesterol ($p < 0.001$). CPI explained 4.3% of the variability in waist circumference and 5.2% of the variability in systolic BP. Similar findings were observed for subjects with CAL ≥6 mm. In general, subjects with CPI codes 3–4 or CAL ≥4–5 mm were associated with more positive components among those included in the definition of MetS.

The association between the different MetS definitions, their individual components and severe periodontitis (defined as CPI = 4 or CAL ≥6 mm) are presented in Tables 4 and 5. After adjusting for sex, age, smoking habit, educational level and toothbrushing frequency, in those subjects with CPI = 4 (Table 4), the prevalence of MetS was higher (OR = 1.41; 95% CI 1.10–1.81; p = 0.007, with IDF definition;

TABLE 1 Demographic and clinical characteristics of study participants by Metabolic Syndrome (MetS) diagnosis

		NCEP-ATP III Criteria			IDF Criteria		
Characteristic	All	Without MetS	With MetS	*p*	Without Met	With MetS	*p*
Number of participants	4353 (100%)	3985 (91.6%)	368 (8.5%)		3646 (83.8%)	707 (16.2%)	
Age group (years)							
<25	390 (9.0%)	379 (9.5%)	11 (3.0%)	<0.001	376 (10.3%)	14 (2.0%)	<0.001
25–34	1374 (31.6%)	1316 (33.0%)	58 (15.8%)		1279 (35.1%)	95 (13.4%)	
35–44	1289 (29.6%)	1191 (29.9%)	98 (26.6%)		1101 (30.2%)	188 (26.6%)	
45–54	877 (20.2%)	765 (19.2%)	112 (30.4%)		639 (17.5%)	238 (33.7%)	
≥55	423 (9.7%)	334 (8.4%)	89 (24.2%)		251 (6.9%)	172 (24.3%)	
Male sex, %	2623 (60.1%)	2345 (58.9%)	278 (75.6%)	<0.001	2091 (57.4%)	532 (75.3%)	<0.001
Smoking habit, %							
Non smoker	2081 (51.1%)	1939 (52.0%)	142 (41.3%)	<0.001	1815 (53.3%)	266 (39.7%)	<0.001
Former smoker	608 (14.9%)	534 (14.3%)	74 (21.5%)		454 (13.3%)	154 (23.0%)	
Smoker ≤10 cig/day	686 (16.8%)	634 (17.0%)	52 (15.1%)		578 (17.0%)	108 (16.1%)	
Smoker >10 cig/day	701 (17.2%)	625 (16.8%)	76 (22.1%)		559 (16.4%)	142 (21.2%)	
Occupation, %							
White-collar	2297 (52.8%)	2123 (53.2%)	180 (47.6%)	0.036	1947 (53.4%)	350 (49.5%)	0.058
Blue-collar	2056 (47.2%)	1871 (46.9%)	198 (52.4%)		1699 (46.6%)	357 (50.5%)	
Country of origin, %							
Spain	3752 (89.4%)	3430 (89.2%)	322 (91.5%)	0.185	3121 (88.6%)	631 (93.3%)	<0.001
Other countries	445 (10.6%)	415 (10.8%)	30 (8.5%)		400 (11.4%)	45 (6.7%)	
Education, %							
Primary School	1044 (24.9%)	916 (23.8%)	128 (36.8%)	<0.001	792 (22.5%)	252 (37.6%)	<0.001
Secondary School	1765 (42.1%)	1625 (42.2%)	140 (40.2%)		1509 (42.8%)	256 (38.2%)	
University	1388 (33.1%)	1308 (34.0%)	80 (22.3%)		1225 (34.7%)	163 (24.3%)	
Net income (monthly), %							
<1200 €	1201 (31.1%)	1108 (31.3%)	93 (29.6%)	0.419	1026 (31.6%)	175 (28.7%)	0.082
1201–3600 €	2210 (57.3%)	2020 (57.0%)	190 (60.5%)		1836 (56.5%)	374 (61.3%)	
>3600€	447 (11.6%)	416 (11.7%)	31(9.9%)		386 (11.9%)	61 (10.0%)	
BMI (kg/m^2)							
Normal	1979 (45.5%)	1944 (48.8%)	35 (9.5%)	<0.001	1930 (52.9%)	49 (6.9%)	<0.001
Overweight	1657 (38.1%)	1520 (38.1%)	137 (37.2%)		1318 (36.2%)	339 (48.0%)	
Obese	717 (16.5%)	521 (13.1%)	196 (53.3%)		398 (10.9%)	319 (45.1%)	
Waist circumference (cm)	87.7 ± 12.9	86.3 ± 12.0	102.7 ± 12.2	<0.001	85.1 ± 11.7	101.0 ± 10.1	<0.001
Triglycerides (mg/dl)	105.0 ± 80.2	97.1 ± 65.6	219.1 ± 151.7	<0.001	92.7 ± 62.7	176.3 ± 122.1	<0.001
HDL (mg/dl)	59.1 ± 14.7	59.9 ± 14.6	47.8 ± 11.6	<0.001	60.2 ± 14.7	52.7 ± 13.1	<0.001
Systolic/diastolic blood pressure (mm Hg)	121 ± 17/75 ± 11	119 ± 16/74 ± 11	138 ± 16/86 ± 11	<0.001	118 ± 15/74 ± 10	134 ± 17/84 ± 11	<0.001
Fasting plasma glucose (mg/dl)	86.6 ± 15.3	85.3 ± 12.5	105.4 ± 31.0	<0.001	84.5 ± 12.3	98.8 ± 23.2	<0.001

Abbreviations: BMI, body mass index; cig, cigarette; HDL, high-density lipoprotein; IDF, International Diabetes Federation; NCEP-ATP, National Cholesterol Education Program Adult Treatment Panel III.

TABLE 2 Periodontal measures and oral health behaviours of study participants by Metabolic Syndrome (MetS) diagnosis

		NCEP-ATP III Criteria			IDF Criteria		
Characteristic	All	Without MetS	With MetS	p	Without MetS	With MetS	p
CPI: Percentage of subjects who have as highest code (%)							
Code 0	178 (4.1%)	167 (4.2%)	11 (3.0%)	<0.001	165 (4.5%)	13 (1.8%)	<0.001
Code 1	343 (7.9%)	330 (8.3%)	13 (3.5%)		320 (8.9%)	23 (3.3%)	
Code 2	2187 (50.2%)	2036 (51.1%)	151 (41.0%)		1883 (51.7%)	304 (43.0%)	
Code 3	1224 (28.1%)	1100 (27.6%)	124 (33.7%)		979 (26.9%)	245 (34.7%)	
Code 4	421 (9.7%)	352 (8.8%)	69 (18.8%)		299 (8.2%)	122 (17.3%)	
Percentage of subjects with CAL (%)							
0–3 mm	3455 (79.37%)	3211 (80.6%)	244 (66.3%)	<0.001	2988 (82.0%)	467 (66.1%)	<0.001
4–5 mm	587 (13.5%)	516 (13.0%)	71 (19.3%)		438 (12.0%)	129 (21.1%)	
≥6 mm	311 (7.1%)	258 (6.5%)	53 (14.4%)		220 (6.0%)	91 (12.9%)	
Number of teeth	25.6 ± 3.8	25.8 ± 3.5	23.8 ± 5.6	<0.001	25.9 ± 3.4	24.0 ± 5.0	<0.001
Dental visits							
<Once a year	1985 (47.7%)	1809 (47.5%)	176 (50.1%)	.346	1633 (46.8%)	352 (52.4%)	0.009
≥Once a year	2173 (52.3%)	1998 (52.5%)	175 (49.9%)		1853 (53.2%)	320 (47.6%)	
Number of teeth	25.6 ± 3.8	25.8 ± 3.5	23.8 ± 5.6	<.001	25.9 ± 3.4	24.0 ± 5.0	<.001
Toothbrushing frequency							
<twice a day	1735 (39.8%)	1533 (38.5%)	202 (54.9%)	<0.001	1353 (37.1%)	382 (54.0%)	<0.001
≥Twice a day	2618 (60.1%)	2452 (61.5%)	166 (45.1%)		2293 (62.9%)	325 (46.0%)	
Dental floss usage							
Yes	830 (20.0%)	767 (20.2%)	63 (18.1%)	0.336	714 (20.5%)	116 (17.5%)	0.076
No	3315 (80.0%)	3029 (79.8%)	286 (82.0%)		2768 (79.5%)	547 (82.5%)	
Mouth rinse usage							
Yes	1606 (36.9%)	1468 (36.8%)	138 (37.5%)	0.801	1341 (36.8%)	265 (37.5%)	0.723
No	2747 (63.1%)	2517 (63.2%)	230 (62.5%)		2305 (63.2%)	442 (62.5%)	

Abbreviations: NCEP-ATP, National Cholesterol Education Program Adult Treatment Panel III; IDF, International Diabetes Federation; CPI, Community Periodontal Index; CAL, clinical attachment level.

and OR=1.58; 95% CI 1.16–2.16; p = 0.004, with ATP III criteria). When severe periodontitis was defined as CAL ≥6 mm (Table 5), similar results were observed (OR = 1.37; 95% CI 1.04–1.82; p = 0.027, with IDF definition; and OR = 1.57; 95% CI 1.11–2.21; p = 0.010, with ATP III criteria). Among the individual components, only high BP was associated with severe periodontitis (OR = 1.44; 95% CI 1.14–1.81; p = 0.002, for CPI = 4; and OR = 1.94; 95% CI 1.49–2.53; p ≥ 0.001, for CAL ≥ 6 mm), although statistically significant associations were observed between the number of positive MetS components (two or more) and severe periodontitis (OR=1.41; 95% CI 1.12–1.77; p = 0.003, for CPI = 4; and OR = 1.47; 95% CI 1.13–1.90; p = 0.004, for CAL ≥6 mm). The analysis of the relationship of MetS with tooth loss showed that MetS, as determined by the IDF criteria, was associated with tooth loss, with an OR of 1.89 (95% CI 1.05–3.40; p = 0.033), after adjusting for confounders.

After stratifying by sex and adjusting for potential confounders, females showed greater ORs of having MetS, independently of the definition (Tables 4 and 5). For the IDF criteria, females with CPI = 4 presented an OR = 2.20 (95% CI 1.31–3.62; p = 0.003) of suffering MetS. No association was found between MetS and periodontal status in males, with the exception of subjects with CPI=4, and according to the NCEP-ATP III definition (OR = 1.44; 95% CI 1.01–2.04; p = 0.043).

Table S2 depicts the prevalence of the different metabolic-obesity phenotypes in the studied population. Out of the 4353 subjects, 66.1% (n = 2879) were MHNO, 17.4% (n = 757) were MNHNO and among those obese there was a higher percentage of MHO (9.1%; n = 394)) than MNHO (7.4%; n = 323). Subjects without metabolic health, either obese or not, were more likely to be older, smokers and to present a worse periodontal condition. The percentage of subjects with deep periodontal pockets (6 mm, CPI code 4)) was significantly higher (p ≥ 0.001) in the metabolically unhealthy groups (14.0% for MNHNO and 19.5% for MNHO) when compared to the metabolically healthy ones (7.4% for MHNO and 10.2% for MHO), independently of the body mass index category. Similar findings were observed when CAL was used as the periodontal criterion.

The association between periodontal measures (CPI or CAL) and body size phenotypes is presented in Table 6. After adjusting for confounders, the results of the multinomial logistic regression analysis showed that severe periodontitis was significantly associated

TABLE 3 Characteristics of the participants for the Metabolic Syndrome (MetS) components (according to the IDF definition) by Community Periodontal Index (CPI)

	Percentage of subjects who have as highest code						
	Code 0 (%)	Code 1 (%)	Code 2 (%)	Code 3 (%)	Code 4 (%)	*p* value	Adjusted R^2
Abdominal obesity (*n*; %)	71 (39.9%)[a]	114 (33.2%)	907 (41.2%)	629 (51.4%)*	240 (57.0%)*	<0.001	
Waist circumference (cm; mean ± *SD*)	84.2 ± 13.3	82.2 ± 11.9	86.6 ± 12.9	90.0 ± 12.3	92.6 ± 12.0	<0.001	0.043
Men	91.1 ± 10.7	89.9 ± 11.4	92.1 ± 11.4	94.1 ± 10.8	95.3 ± 10.8	<0.001	0.014
Women	77.3 ± 12.0	76.7 ± 8.7	79.1 ± 10.9	82.2 ± 10.9	84.3 ± 11.8	<0.001	0.033
Hypertriglyceridaemia (*n*; %)	35 (20.6%)[a]	44 (13.6%)	490 (23.4%)	371 (31.4%)*	152 (37.6%)*	<0.001	
Triglycerides (mg/dl; mean ± *SD*)	87.0 ± 49.5	82.1 ± 42.5	98.5 ± 67.1	115.3 ± 88.0	134.9 ± 126.0	<0.001	0.029
High blood pressure (*n*; %)	41 (24.1%)[a]	69 (20.9%)	606 (28.7%)	489 (41.1%)*	217 (52.9%)*	<0.001	
Systolic/diastolic blood pressure (mm Hg; mean ± *SD*)	116 ± 15/73 ± 10	115 ± 16/71 ± 10	119 ± 16/74 ± 11	124 ± 17/77 ± 11	129 ± 18/79 ± 11	<0.001	0.052/0.035
Hyperglycaemia (*n*; %)	24 (14.4%)[a]	23 (7.1%)	349 (16.8%)	201 (17.1%)	89 (22.0%)*	<0.001	
Fasting blood glucose (mg/dl; mean ± *SD*)	83.7 ± 9.9	81.8 ± 9.2	86.0 ± 13.3	87.4 ± 15.3	92.4 ± 25.4	<0.001	0.024
Low HDL cholesterol (*n*,%)	14 (8.3%)[a]	38 (11.8%)	289 (13.7%)	220 (18.7%)*	91 (22.7%)*	<.001	
HDL (mg/dl; mean ± *SD*)	61.9 ± 13.9	62.4 ± 15.2	59.3 ± 14.9	57.9 ± 14.3	57.6 ± 14.2	<.001	0.007
Men	56.2 ± 12.1	54.1 ± 13.1	53.6 ± 11.6	54.0 ± 12.2	54.3 ± 12.4	0.375	
Women	67.1 ± 13.5	68.5 ± 13.7	67.2 ± 15.3	65.6 ± 15.1	68.4 ± 14.2	0.152	
Number of positive MetS components (*n*, %)							
0	104 (58.4%)[a]	220 (64.1%)	1100 (50.3%)	473 (38.6%)*	127 (30.2%)*	<0.001	
1	45 (25.3%)[a]	82 (23.9%)	607 (27.8%)	390 (31.9%)*	125 (29.7%)		
2	20 (11.2%)[a]	33 (9.6%)	339 (15.5%)	223 (18.2%)*	99 (23.5%)*		
3	7 (3.9%)[a]	6 (1.8%)	115 (5.3%)	107 (8.7%)*	54 (12.8%)*		
4	2 (1.1%)[a]	2 (0.6%)	26 (1.2%)	31 (2.5%)*	16 (3.8%)*		

Abbreviations: IDF, International Diabetes Federation; *SD*, standard deviation; HDL, high-density lipoprotein.

[a]Reference category.

*Statistically significant difference when compared with the reference category ($p < 0.01$).

TABLE 4 Association between the different definitions of Metabolic Syndrome (MetS) and its individual components with severe periodontitis (defined as CPI code 4)

	Total		Male gender		Female gender	
	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
MetS (IDF definition)	**1.41 (1.10–1.81)**	0.007	1.25 (0.94–1.66)	0.128	**2.20 (1.31–3.62)**	0.003
Abdominal obesity	1.19 (0.95–1.49)	0.126	1.08 (0.83–1.40)	0.563	1.50 (0.96–2.34)	0.075
Hypertriglyceridaemia	1.17 (0.93–1.48)	0.188	1.09 (0.83–1.43)	0.523	1.45 (0.88–2.38)	0.148
High blood pressure	**1.44 (1.14–1.81)**	0.002	1.28 (0.99–1.66)	0.064	**2.18 (1.39–3.41)**	0.001
Hyperglycaemia	1.10 (0.84–1.44)	0.490	1.04 (0.77–1.42)	0.789	1.25 (0.70–2.25)	0.451
Low HDL cholesterol	**1.40 (1.06–1.83)**	0.015	**1.60 (1.18–2.17)**	0.003	0.92 (0.51–1.68)	0.788
≤1 component vs ≥2 components	**1.41 (1.12–1.77)**	0.003	1.26 (0.95–1.69)	0.114	**1.58 (1.03–2.41)**	0.034
MetS (NCEP-ATP III definition)	**1.58 (1.16–2.16)**	0.004	**1.44 (1.01–2.04)**	0.043	**2.33 (1.24–4.39)**	0.009
Abdominal obesity	1.19 (0.93–1.52)	0.174	1.16 (0.87–1.56)	0.316	1.22 (0.77–1.93)	0.388
Hypertriglyceridaemia	1.13 (0.89–1.45)	0.317	1.03 (0.78–1.36)	0.817	1.55 (0.91–2.66)	0.109
High blood pressure	**1.43 (1.14–1.79)**	0.002	1.26 (0.97–1.62)	0.080	**2.27 (1.45–3.54)**	<.001
Hyperglycaemia	**1.39 (1.00–1.94)**	0.052	1.41 (0.97–2.06)	0.071	1.22 (0.59–2.52)	0.595
Low HDL cholesterol	1.33 (0.93–1.90)	0.118	**1.64 (1.08–2.49)**	0.020	0.84 (0.40–1.79)	0.659
≤1 component vs. ≥2 components	**1.39 (1.10–1.74)**	0.005	1.28 (0.96–1.72)	0.090	1.48 (0.96–2.28)	0.075

Bold values merely corresponds with those with *p* values <0.05.

Abbreviations: Adjusted Model: Adjusted for sex, age, smoking habit, educational level and toothbrushing frequency. CPI, Community Periodontal Index; NCEP-ATP, National Cholesterol Education Program Adult Treatment Panel III; IDF, International Diabetes Federation; HDL, high-density lipoprotein; OR, odds ratio; CI, confidence interval.

with metabolically unhealthy subjects, particularly if they were obese (OR = 1.78; 95% CI 1.28–2.49; p = 0.001, for CPI = 4; and OR = 1.49; 95% CI 1.02- 2.20; p = 0.041 for CAL ≥ 6 mm), but no association with metabolically healthy obese subjects was observed.

4 | DISCUSSION

The results of the present study show a significant association between a worse periodontal condition and MetS, as defined using widely accepted criteria.

In the present investigation, the prevalence of MetS was 8.5%, according to NCEP-ATP III definition and 16.2%, using the IDF criteria. This higher prevalence using the definition of the IDF is in agreement with the findings from other epidemiological studies (Herath et al., 2018). In spite of these differences in MetS prevalence depending on the two definitions used, the association between MetS and periodontitis was clear and consistent with both. Patients with severe periodontitis (defined as either CPI = 4 or CAL ≥ 6 mm) presented a risk for suffering MetS more than two times higher than the one for patients with a better periodontal condition. Furthermore, when the components of the syndrome where evaluated separately, all showed a clear tendency towards a significant association between a worse periodontal condition and high prevalence of cardiometabolic abnormalities. As an example, the prevalence of high BP in the group of patients diagnosed with CPI = 4 or CAL ≥ 6 mm doubled the prevalence among subjects with CPI = 0 or CAL = 0–3 mm (for CPI, 52.9% versus 24.1%, respectively; for CAL, 60.3% versus 29.1%, respectively). Similar observations were found for lipids, glycaemic levels and abdominal obesity. These findings are in agreement with previous investigations in other population-based studies, such as the NHANES III in the United States, where the prevalence of MetS was two times higher among patients with moderate or severe periodontitis when compared with subjects with healthy or mild disease (D'Aiuto et al., 2008).

After adjusting for potential confounders (sex, age, smoking status, educational level and toothbrushing frequency), high BP was the individual component of MetS that demonstrated the strongest association with severe periodontitis (defined either with CAL or CPI), especially among women. A recent systematic review with meta-analysis showed an OR = 1.49 (95% CI 1.09–2.50) for the association between severe periodontitis and high BP (Munoz Aguilera et al., 2020). Moreover, recent evidence from a cross-sectional survey among treated hypertensive adults have suggested that periodontitis may be associated with unsuccessful antihypertensive treatment, as periodontally healthy subjects presented a mean systolic BP 2.3−−3 mmHg lower, when compared with periodontitis patients (Pietropaoli et al., 2018). Further intervention trials are needed to clarify the benefits of periodontal treatment upon BP, but on the basis of the available evidence, a positive effect may be expected.

Besides high BP, the main contributors to the association between periodontitis and MetS reported in the literature are low

TABLE 5 Association between the different definitions of Metabolic Syndrome (MetS) and its individual components with severe periodontitis (defined as CAL ≥6 mm)

	Total		Male gender		Female gender	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
MetS (IDF definition)	**1.37 (1.04–1.82)**	0.027	1.29 (0.95–1.77)	0.105	1.77 (0.92–3.39)	0.085
Abdominal obesity	1.16 (0.89–1.51)	0.281	**1.49 (1.11–2.00)**	0.008	1.44 (0.83–2.51)	0.196
Hypertriglyceridaemia	1.17 (0.93–1.48)	0.188	1.02 (0.76–1.37)	0.898	1.75 (0.97–3.18)	0.065
High blood pressure	**1.94 (1.49–2.53)**	<.001	**1.90 (1.41–2.57)**	<.001	**2.24 (1.29–3.88)**	0.004
Hyperglycaemia	1.29 (0.96–1.74)	0.094	1.13 (0.81–1.58)	0.477	**1.99 (1.04–3.81)**	0.037
Low HDL cholesterol	1.33 (0.97–1.80)	0.073	**1.57 (1.12–2.20)**	0.009	0.61 (0.25–1.45)	0.260
≤1 component vs ≥2 components	**1.47 (1.13–1.90)**	0.004	**1.88 (1.31–2.68)**	0.001	**1.94 (1.13–3.31)**	0.016
MetS (NCEP-ATP III definition)	**1.57 (1.11–2.21)**	0.010	1.29 (0.87–1.90)	0.209	**3.63 (1.80–7.20)**	<.001
Abdominal obesity	1.19 (0.93–1.52)	0.174	1.12 (0.81–1.55)	0.493	1.64 (0.94–2.85)	0.080
Hypertriglyceridaemia	1.17 (0.88–1.54)	0.276	0.99 (0.73–1.35)	0.960	**2.18 (1.18–4.03)**	0.013
High blood pressure	**1.96 (1.51–2.54)**	<.001	**1.92 (1.43–2.57)**	<.001	**2.34 (1.35–4.06)**	0.002
Hyperglycaemia	**1.49 (1.03–2.15)**	0.034	1.35 (0.89–2.04)	0.160	1.91 (0.87–4.17)	0.105
Low HDL cholesterol	1.35 (0.90–2.02)	0.148	**1.63 (1.03–2.58)**	0.038	0.88 (0.34–2.26)	0.789
≤1 component vs. ≥2 components	**1.57 (1.21–2.03)**	0.001	**1.75 (1.23–2.47)**	0.002	**2.09 (1.19–3.68)**	0.011

Bold values merely corresponds with those with *p* values <0.05.

Abbreviations: Adjusted Model: Adjusted for sex, age, smoking habit, educational level and toothbrushing frequency; CAL: clinical attachment level; NCEP-ATP: National Cholesterol Education Program Adult Treatment Panel III; IDF: International Diabetes Federation; HDL: high-density lipoprotein; OR: odds ratio; CI: confidence interval.

TABLE 6 Association between periodontal measures (CPI and CAL) and body size phenotypes

	CPI Code 4				CAL ≥ 6 mm			
	Crude model		Adjusted model		Crude model		Adjusted model	
Body size phenotype	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
MHNO[a]								
MHO	**1.42 (1.00–2.03)**	0.053	1.03 (0.71–1.51)	0.862	**1.52 (1.01–2.29)**	0.044	1.03 (0.67–1.59)	0.908
MNHNO	**2.05 (1.60–2.63)**	<.001	**1.31 (1.00–1.71)**	0.047	**2.52 (1.92–3.32)**	<.001	**1.46 (1.09–1.96)**	0.011
MNHO	**3.05 (2.24–4.15)**	<.001	**1.78 (1.28–2.49)**	0.001	**2.76 (1.92–3.97)**	<.001	**1.49 (1.02–2.20)**	0.041

Adjusted Model: Adjusted for sex, age and smoking habit. Bold values merely corresponds with those with *p* values <0.05.

Abbreviations: CAL, clinical attachment level; CI, confidence interval; MHNO: Metabolically Healthy Non-Obese; MHO: Metabolically Healthy Obese; MNHNO: Metabolically Non-Healthy Non-Obese; MNHO: Metabolically Non-Healthy Obese. CPI, Community Periodontal Index; OR, odds ratio.

[a]Reference category.

levels of HDL cholesterol and elevated fasting glucose (Alhabashneh et al., 2015; D'Aiuto et al., 2008; Shimazaki et al., 2007; Tu et al., 2013). According to the IDF criteria, elevated fasting plasma glucose includes both impaired fasting glucose (i.e. prediabetes) and values characteristic of diabetes mellitus. Previous studies have reported controversial data regarding the association between periodontitis and prediabetes, with some studies demonstrating a significant association {Saito, 2004 #343}{Zadik, 2010 #798}{Choi, 2011 #135}{Arora, 2014 #44}, but with other studies performed in European populations, and using different measures of periodontitis (e.g. mean probing pocket depth, percentage of sites with CAL ≥ 4 mm), reporting no significant differences in the periodontal condition of subjects with or without prediabetes {Noack, 2000 #66}{Kowall, 2015 #63}. Similar findings from the WORALTH study have been previously published by our research group, proving a significant association between periodontitis and diabetes mellitus, but failing to demonstrate an association between periodontitis and prediabetes {Montero, 2019 #799}. In the present manuscript, when pooling both

conditions under the term "Hyperglycaemia", no significant association could be found with CPI code 4 or CAL ≥ 6 mm.

Abdominal obesity, despite being a crucial component in the definition of MetS, does not seem to play a key role in the association with periodontitis. Most cross-sectional studies on MetS patients report either no association between obesity and periodontitis (Alhabashneh et al., 2015; Benguigui et al., 2010; D'Aiuto et al., 2008), or associations of small magnitude and no statistically significant (Timonen et al., 2010). These findings are in agreement with the results of the present study. Furthermore, when patients where categorized by body size phenotype, obesity alone, with no concomitant cardiometabolic abnormalities (MHO), did not show association with periodontitis, while subjects presenting altered metabolic profiles showed a greater tendency towards a worse periodontal condition, independently of being obese or with normal weight (MNHNO and MNHO, respectively). These observations suggest the possible role of the low-grade systemic inflammatory status reported in severe periodontitis as a contributor in the pathophysiology of certain metabolic alterations, such as the increase in plasma glucose levels and abnormalities in the lipid profiles, even in the absence of large fat deposits. In any case, it should be noted that all anthropometric measures (WC, BMI, etc.) are not necessarily indicative of the amount of visceral fat, and therefore, in order to evaluate the potential association between adiposity and periodontitis, imaging techniques such as magnetic resonance should be used in the future.

The association between periodontitis and MetS seems to be stronger in women, as reported in different population-based studies, with ORs ranging between 1.52 and 3.60 (Andriankaja et al., 2010; Furuta et al., 2013; Tu et al., 2013). Our findings support this gender differences, with significant higher ORs for the association between MetS and periodontitis for female patients, according to both definitions. Some authors have hypothesized that female patients with MetS have a higher systemic inflammatory status (represented by higher levels of C-reactive protein, CRP) than males (Saltevo et al., 2008), and that the protective role of sex hormones against body fat distribution and insulin activity may be diminished by the effect of certain pro-inflammatory cytokines (Alpizar & Spicer, 1994), which are usually in higher levels in severe periodontitis patients (Higashi et al., 2009). Further research is needed to elucidate these mechanisms explaining the significant association between MetS and periodontitis in women.

When assessing the evaluated oral health behavioural factors, a potential protective role of regular toothbrushing was observed, with approximately 10% less probability of being diagnosed with MetS among subjects reporting to brush their teeth twice a day. Similar observations have been made by other authors, reporting an OR = 21.82 for suffering MetS when subjects reported toothbrushing frequencies lower than twice per day (Thanakun et al., 2014). The potential preventive role of oral hygiene on the onset of metabolic disorders, together with the already established benefits of treating periodontitis in patients with diabetes mellitus (D'Aiuto et al., 2018), advocate for, at least, a recommendation for regular periodontal examinations and oral hygiene promotion in subjects with MetS.

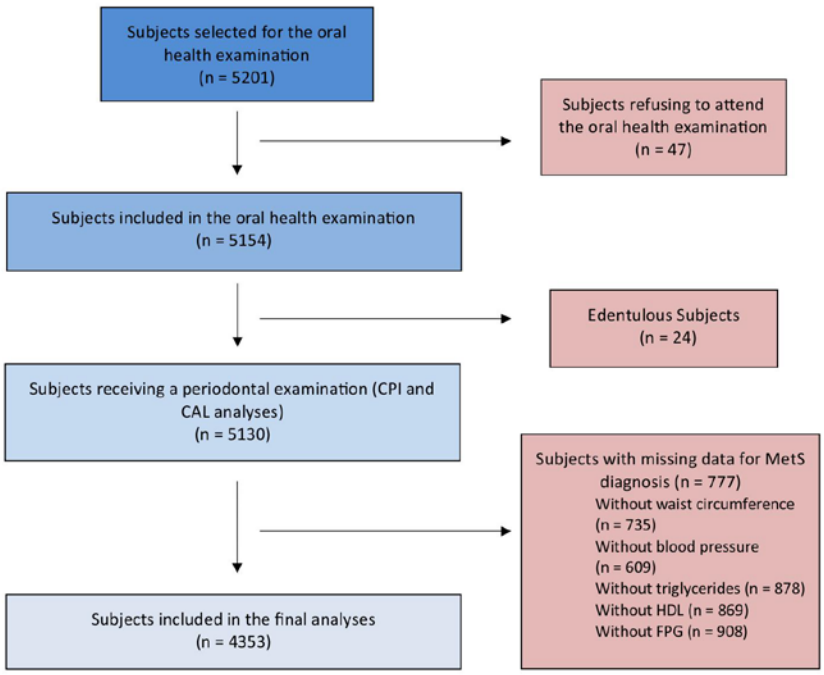

FIGURE 1 Flowchart of the subjects included/excluded in the WORALTH study and in the presence of Metabolic Syndrome (MetS) analysis. CAL, Clinical Attachment Level; CPI, Community Periodontal Index; FPG, Fasting Plasma Glucose); HDL, High-Density Lipoprotein

Different limitations in this investigation should be acknowledged. Firstly, due to the cross-sectional nature of the present study, the direction of the association, or a possible causative role, cannot be established. Secondly, since the Spanish employed population was the target group, only subjects aged between 16 and 65 were included, which may limit the external validity. Furthermore, some of the patients (n = 777) had to be excluded as they did not present enough data to perform a diagnosis of MetS. And thirdly, the periodontal assessment based on the WHO recommendation, consisting on a partial-mouth examination, may underestimate the levels of disease.

Despite the referred limitations of the present investigation, a statistically significant association between metabolic syndrome and a worse periodontal condition has been observed in the working population in Spain, being high blood pressure the component with the strongest role. This association was independent of BMI and other confounders such as age, sex or toothbrushing frequency. However, further research is needed through observational longitudinal studies or intervention trials in order to determine the possible causative relationship between MetS and periodontitis, as well as the benefits derived from periodontal treatment in the cardiometabolic risk of MetS patients.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this research.

DATA AVAILABILITY STATEMENT

Data availability will be considered by the authors upon request.

ORCID

Eduardo Montero https://orcid.org/0000-0003-2525-8529
Mariano Sanz https://orcid.org/0000-0002-6293-5755
David Herrera https://orcid.org/0000-0002-5554-2777

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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STUDY #3

Montero E, López M, Vidal H, Martínez M, Virto L, Marrero J, Herrera D, Zapatero A, Sanz M. (2020) Impact of periodontal therapy on systemic markers of inflammation in patients with metabolic syndrome: A randomized clinical trial. *Diabetes, Obesity and Metabolism.* DOI: 10.1111/jcpe.13353

Impact of periodontal therapy on systemic markers of inflammation in patients with metabolic syndrome: A randomized clinical trial

Aim: To determine the impact of periodontal treatment on systemic markers of inflammation in patients with metabolic syndrome (MetS) and periodontitis.

Materials and Methods: In this parallel-arm, double-blind, randomized controlled clinical trial, 63 patients with MetS and severe periodontitis were randomly assigned to receive either intensive periodontal treatment (IPT; scaling and root planing plus azithromycin 500 mg every day for 3 days) or minimal periodontal treatment (MPT; supragingival professional mechanical plaque removal plus a placebo). The primary outcome was the impact of the tested interventions on high-sensitivity C-reactive protein (hs-CRP) serum levels at 6 months. As secondary outcomes, differences in the levels of cytokines, markers of prothrombotic states, carbohydrate and lipid metabolism, as well as blood pressure, were measured at 3 and 6 months after therapy.

Results: The intention-to-treat population consisted of 63 subjects randomly assigned to either the MPT (n = 31) or the IPT (n = 32) group. At baseline, mean hs- CRP was 3.9 mg/L (standard deviation [SD] = 2.9) and 3.9 mg/L (SD = 3.4), respectively, and no significant differences in cardiometabolic risk profiles were detected between the groups. Adjusting for baseline hs-CRP, sex, age, smoking status and body mass index, hs-CRP at 6 months was 1.2 mg/L (95% CI 0.4; 2.0; P = .004) lower in the IPT group than in the MPT group. In the secondary outcomes, significant reductions in IL-1β, TNF-α, HbA1c and blood pressure were observed in the IPT group at 3 months compared with the MPT group.

Conclusion: Effective periodontal treatment significantly reduced hs-CRP after 6 months in patients with MetS and severe periodontitis. Periodontal therapy might be useful to reduce cardiovascular risk in these patients.

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ORIGINAL ARTICLE

WILEY

Impact of periodontal therapy on systemic markers of inflammation in patients with metabolic syndrome: A randomized clinical trial

Eduardo Montero DMD[1,2] | Mercedes López DMD[1,2] | Honorato Vidal DMD[2] | María Martínez DMD[2] | Leire Virto[1] | Jorge Marrero MD[3] | David Herrera DMD[1,2] | Antonio Zapatero MD[3] | Mariano Sanz MD[1,2]

[1]ETEP (Etiology and Therapy of Periodontal and Peri-implant Diseases) Research Group, University Complutense, Madrid, Spain

[2]Section of Graduate Periodontology, University Complutense, Madrid, Spain

[3]Internal Medicine Department, Fuenlabrada Hospital, Madrid, Spain

Correspondence
Eduardo Montero, Faculty of Odontology, University Complutense of Madrid, Plaza de Ramón y Cajal s/n (Ciudad Universitaria), 28040 Madrid, Spain.
Email: eduardomonterosolis@ucm.es

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Abstract

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KEYWORDS

diabetes, insulin resistance, metabolic syndrome, periodontitis, systemic inflammation

1 | INTRODUCTION

Metabolic syndrome (MetS) is a cluster of medical conditions associated with the risk of developing atherosclerotic cardiovascular disease (CVD).[1] It is characterized by central obesity (defined by waist circumference [WC]), impaired glucose regulation, high blood pressure and dyslipidaemia (i.e. low high-density lipoprotein [HDL] cholesterol and/or elevated triglycerides).[2] MetS is not a disease in itself, but it is a serious health condition and a major global public health concern that affects ~ 10%-40% of adults in developed countries, in parallel with the rise in the prevalence of obesity.[3] In the United States, its prevalence has increased from 28% in 1988-1994 to 34% in 1994-2004, according to the National Health and Nutrition Examination Surveys.[4]

Periodontitis is a highly prevalent chronic inflammatory disease characterized by the destruction of tooth-supporting tissues, eventually leading to tooth loss and significantly impairing the patient's quality of life. In fact, severe periodontitis is one of the most prevalent diseases worldwide, with an age-standardized prevalence of 9.8% and with ~ 796 million cases.[5] Several epidemiological studies and systematic reviews have consistently shown an association between periodontitis and an increased risk of future atherosclerotic cardiovascular events, independent of traditional risk factors (e.g. smoking, diabetes, hypertension and hypercholesterolaemia).[6] This association has been explained by the systemic effect of this infectious disease, evidenced by a significant increase in high-sensitivity C-reactive protein (hs-CRP) and other biomarkers of systemic inflammation, which may contribute to the overall inflammatory burden and to an increased risk of atherogenesis and hyperglycaemia.[7,8]

A recent systematic review with meta-analysis has shown evidence of a potential association between periodontitis and MetS.[9] This association may be explained by the shared chronic state of systemic inflammation in both conditions, resulting in oxidative stress and insulin resistance.[10,11] This bi-directional association may be explained by the increase of systemic inflammation as a consequence of periodontitis, which may act as a contributor to the development of MetS,[12] while the low-grade systemic inflammation characteristic of obesity and impaired glucose regulation may negatively affect the periodontal status.[13]

The possible effect of periodontal treatment on systemic inflammation has been reported in several studies assessing the levels of inflammatory markers after treatment, showing significant reductions in interleukin (IL)-6 and hs-CRP.[14,15] However, this systemic impact has not been consistent and the variability in the reported outcomes has been attributed to inclusion of patients without clear measures of exposure, based on clinical or radiographic surrogates, rather than on the actual infectious burden, which may not reflect the degree of chronic inflammatory exposure.[16] Also, the selection of patients may not have included those with a relevant risk of CVD.[17]

It was, therefore, the purpose of this parallel-arm, double-blind, randomized controlled clinical trial, to determine whether the treatment of periodontitis in patients with MetS could reduce the cardiometabolic risk, measured by means of (a) markers of systemic inflammation and prothrombotic states (hs-CRP, α-1 antitrypsin and fibrinogen levels), (b) proinflammatory cytokine profiles, and (c) markers of carbohydrate and lipid metabolism.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

This study was designed as a parallel-group, double-blind, single-centre, randomized clinical trial (RCT) with a 6-month follow-up. Patients were recruited among those diagnosed with MetS and included in an active programme of cardiovascular risk prevention at the Fuenlabrada University Hospital (Madrid Regional Hospital Network, Fuenlabrada, Madrid, Spain). The study protocol was approved by the institutional ethic committee (Internal Code 12/206, Hospital Clínico de San Carlos, Madrid, Spain) and registered at ClinicalTrials.gov (NCT03960216). This RCT was conducted from November 2012 to June 2018.

Participants were consecutively enrolled in the study if they fulfilled the following criteria:

- Aged 35 to 65 years.
- Diagnosis of MetS according to the criteria established by Alberti et al.[18] in 2009 based on the presence of at least three of the following risk factors:

- Elevated WC (≥94 cm in men, ≥80 cm in women).
- Elevated triglycerides (≥150 mg/dL).
- Reduced HDL cholesterol (<40 mg/dL in males, <50 mg/dL in females).
- Elevated blood pressure (systolic ≥130 and/or diastolic ≥85 mmHg).
- Elevated fasting plasma glucose (FPG) (≥100 mg/dL).

- Presence of at least 16 teeth.
- Diagnosis of stage III-IV generalized periodontitis, according to the criteria of the 2018 European Federation of Periodontology-American Academy of Periodontology classification.[19] These are the most severe forms of periodontitis, showing an attachment loss of ≥5 mm with radiographic bone loss extending to the middle or apical third of the root.
- Presence of at least eight sites with a probing pocket depth (PPD) of ≥6 mm and four sites with a clinical attachment level (CAL) of ≥5 mm, distributed in at least two different quadrants,[20] with the objective of recruiting participants with severe periodontal destruction (defined by CAL) and a high level of inflammation (defined by PPD).

Participants were excluded by the presence of:

- Uncontrolled systemic diseases other than diabetes or hypertension (e.g. chronic kidney disease, chronic lung disease, acute pulmonary disease during the previous 3 months, history of stroke, myocardial infarction, angor pectoris or revascularization during the previous 6 months, neoplasms).
- History of any surgical treatment in the previous 3 months.

- Alcoholism or psychiatric disorders.
- History of systemic antibiotic usage over the previous 3 months.
- Non-surgical periodontal treatment during the previous 6 months, or surgical periodontal treatment over the previous 12 months.

All the patients recruited according to these criteria and willing to participate in the study received verbal and written information. Upon signing the approved consent form, they were referred for periodontal treatment to the Postgraduate Clinic of Periodontology at the Faculty of Odontology (University Complutense of Madrid, Spain).

2.2 | Study interventions

All patients received standardized oral hygiene instructions (consisting of the use of a manual toothbrush and specific inter-dental brushes) and, when indicated, extraction of hopeless teeth. Then patients were randomized to one of the two following groups:

- An intensive periodontal treatment (IPT) test group, which consisted of two sessions (1 week apart) of non-surgical subgingival instrumentation (scaling and root planing [SRP]) under local anaesthesia, with a combination of ultrasonic scalers (Minipiezon Electromedical Systems, Nyon, Switzerland) and hand curettes, together with adjunctive administration of a systemic antibiotic (azithromycin 500 mg every day (q.d.) for 3 days), administered at the last session of SRP.[21]
- A minimal periodontal treatment (MPT) control group, which consisted of two sessions of supragingival professional mechanical plaque removal, eliminating supragingival plaque and calculus with an ultrasonic scaler, without subgingival instrumentation, together with the adjunctive administration of placebo medication (identical capsules containing lactose 500 mg q.d. for 3 days) administered at the last session of SRP.

Both treatment groups received, after the first treatment session, an antiseptic mouth rinse, containing 0.12% chlorhexidine and 0.05% cetylpyridinium chloride (Perio-Aid 0.12%; Dentaid, Barcelona, Spain), to be used twice daily for 14 days. Oral hygiene instructions were reinforced in both groups at the 3- and 6-month visits, but no additional periodontal treatment was provided during the study, unless a subject showed periodontitis progression, defined as the presence of interproximal attachment loss of ≥3 mm in at least two teeth.[22] These patients were withdrawn from the study and immediately received the appropriate periodontal treatment.

2.3 | Randomization and masking

Patients were randomly assigned to receive IPT or MPT (1:1) using a Microsoft Excel-generated list, with a random block size of 20. Allocation concealment was ensured by the use of opaque envelopes, opened at the time of the start of periodontal treatment, by the assigned clinicians (HV for patients 21-63 and EM for patients 1-20). Patients were unaware of the assigned treatment group, and the adjunctive medication (azithromycin or placebo) was provided as externally identical capsules, numbered according to the randomization list. All other investigators (clinical examiners, laboratory staff, staff involved in data collection [MM]) were masked to the group allocation.

2.4 | Study flow

Two trained and calibrated clinical examiners (EM for patients 21-63 and ML for patients 1-20) collected medical histories and carried out a comprehensive oral and periodontal examination, at baseline, and at each follow-up visit (3 and 6 months after therapy). Periodontal variables included full-mouth measurements of PPD, CAL, bleeding on probing (BOP), plaque index (PII)[23] and gingival index (GI),[24] at six sites per tooth, excluding third molars, using a UNC-15 probe (Hu-Friedy, Chicago, IL, USA). Full-mouth periapical radiographs and/or a panoramic radiograph were also taken at baseline. Before the start of the study, clinical examiners were calibrated (presented in the supporting information). Exposure to tobacco smoking was recorded as either current (the number of cigarettes smoked and years of exposure were noted), former (those who had stopped smoking at least 1 year previously) or having never smoked. Measurements of weight, height, WC and blood pressure were carried out at baseline and at the 3- and 6-month examinations. History of any other used medication was collected at baseline and updated at each study visit.

Fasting blood samples were collected at baseline, and at each study visit, into three 7 mL ethylenediaminetetraacetic acid (EDTA)-containing tubes. One EDTA-containing tube was stored at the hospital at −80°C, for later analysis of hs-CRP, α-1 antitrypsin, fibrinogen, white blood cell count (WBC), HbA1c (using high-performance liquid chromatography), FPG, insulin, creatinine and standard lipid fractions (total cholesterol, HDL cholesterol, low-density lipoprotein [LDL] cholesterol and triglycerides). The other two tubes were stored at −80°C for later determination of cytokine profiles (IL-1β, IL-6, IL-18 and tumour necrosis factor [TNF]-α) at the Molecular Biology Laboratory (Faculty of Odontology, University Complutense of Madrid) by means of multiplex assays. At the same time points, microbiological samples were collected before the periodontal examination. Detailed information regarding the laboratory procedures for the multiplex assays and the microbiological analyses is provided in the supporting information.

2.5 | Outcomes

The primary outcome was the evaluation of the differences between the test group and the control group in the mean hs-CRP serum levels at 6 months after the periodontal intervention. As secondary outcomes, the mean levels at 6 months of α-1 antitrypsin, fibrinogen, inflammatory markers (IL-1β, IL-6, IL-8 and TNF-α), HbA1c, FPG, insulin, lipid fractions and blood pressure were compared.

To assess the efficacy of the tested interventions on periodontal health, mean periodontal clinical variablesget (PPD, CAL, BOP, PII and GI) and mean counts, proportions and frequency of detection of target periodontal pathogens (*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Parvimonas micra*, *Campylobacter rectus* and *Fusobacterium nucleatum*) were compared at 3 and 6 months as prespecified secondary outcomes.

Compliance with antibiotic intake and occurrence of adverse events were also registered. Patients exited from the study in the presence of serious adverse effects under the criteria of the medical principal investigator (AZ) or when the criteria for periodontitis recurrence were met.

Homeostasis Model Assessment scores (HOMA2-IR, HOMA2-β and HOMA2-IS) were calculated with a HOMA2 calculator released by the Diabetes Trial Unit, University of Oxford (https://www.dtu.ox.ac.uk/homacalculator/). The concentrations of IL-1β, IL-6, IL-8 and TNF-α were measured using high-sensitivity multiplex map human immunoassays (Millipore, Cat. #HSTCMAG-28SK, Billerica, MA, USA) using a Luminex-200 System Unit with the XY platform (Luminex, Oosterhout, the Netherlands). Detailed information regarding the laboratory procedures for the immunoassays is provided in the supporting information.

2.6 | Statistical methods

The sample size calculation was based on detecting a mean difference between groups of 2 mg/L in serum hs-CRP levels with an estimated standard deviation (SD) of 2.5 mg/L. To detect statistically significant differences ($\alpha = 0.05$) with 80% power, a minimum of 25 subjects per group were needed, which including an expected 20% dropout rate, resulting in a final sample of 30 participants per group.

Data were reported as mean and SD unless otherwise specified (e.g. n [%]). All analyses were performed using the intention-to-treat population and the last observation carried forward approach for missing values. Analyses were repeated for all primary and secondary outcomes using post hoc missing value analyses. In addition, per-protocol analyses were conducted for all outcomes; the estimates derived from these analyses were reported for the primary outcome (hs-CRP) and any secondary or post hoc outcomes if they differed from estimates obtained in the intention-to-treat population.

The primary and secondary outcomes were modelled using multilevel linear regression with the xtmixed command in STATA version 13.1 (StataCorp College Station, TX, USA). The models included the respective baseline measurement, treatment group, study visit (i.e. baseline, 3 and 6 months) and a treatment-time interaction term as explanatory variables (covariates). Additional covariates included the main risk determinants for CVDs and periodontitis (i.e. age and sex) and the main risk factors also linked to a poorer clinical periodontal outcome after treatment (i.e. smoking status and body mass index [BMI]). Where appropriate, natural logarithmic transformation of the data was applied, and a model with random intercepts and unstructured variance-covariance was used. Differences between study groups for primary and secondary outcomes at all study follow-up visits (3 and 6 months) were adjusted for the respective baseline values, whereas the absolute values for each treatment group at a given time point were not.

3 | RESULTS

3.1 | Patient characteristics

From 28 November 2012 to 11 December 2017, 854 subjects with MetS were screened, of whom 765 were excluded because they did not meet the study criteria. Sixty-three (70.8%) of 89 subjects were finally enrolled and randomly assigned to either the test IPT (n = 32) or the control MPT (n = 31) group (Figure 1). Medical treatment and established protocols for these patients did not change throughout the study period, suggesting that the duration of a patient's inclusion period (5 years) could not be considered as a bias in this trial. Three patients (5%) were lost in each group over the 6-month period (three were lost to follow-up at 3 months in the MPT group; one was lost to follow-up at 3 months and two were lost to follow-up at 6 months in the IPT group). These patients did not significantly differ from the included participants with regard to any of the outcome variables under consideration in the study. All of the included subjects were Caucasians. Two major adverse events occurred during the course of the study, one in a patient in the test group (hospitalization for urine infection), and the other in a patient in the control group (acute myocardial infarction). These events were not considered to be related to the study, and they did not prevent either patient from attending the follow-up visits. The occurrence of minor adverse events was similar between both study groups (Table S1).

The cardiometabolic risk profiles at baseline (Table 1) did not show any significant difference between groups. Participants were predominantly men (~ 70% in both groups), aged 50-65 years and mostly had either never smoked or were former smokers (75.9% in the IPT group and 89.3% in the MPT group). Subjects in both groups presented a mean BMI close to class 3 obesity (39.1 kg/m^2 for the IPT group and 38.0 kg/m^2 for the MPT group; $P = .618$) and a mean WC ≈ 120 cm, indicative of prominent visceral fat storage. High hs-CRP levels were observed in both groups, with 53.1% and 54.8% of patients in the IPT and MPT groups, respectively, presenting values of ≥3 mg/L. The percentage of subjects with HbA1c levels of ≥7% were 31.25% and 25.8%, respectively. No differences between groups were observed in medication use at baseline (Table S2) and changes in medication throughout the study were negligible (n = 3 [4.8%], corresponding to an increase in insulin dosage). Nine patients (two in the test group and seven in the control group) received systemic antimicrobials during the study (apart from the one

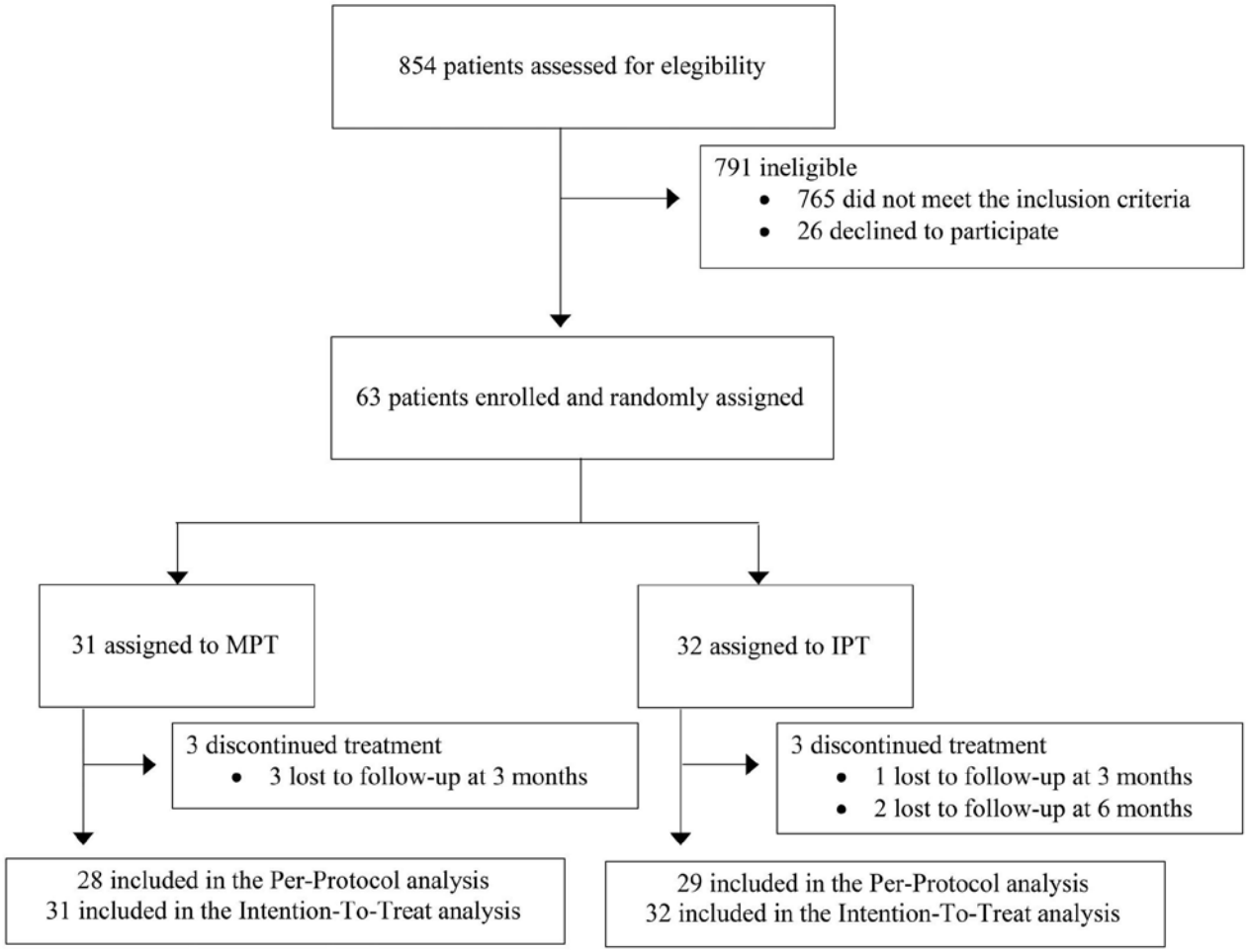

FIGURE 1 Flowchart of the trial

administered in the IPT group), but differences between the groups were not statistically significant ($P = .070$).

3.2 | Clinical and microbiological periodontal outcomes

Periodontal clinical variables were similar at baseline in both groups (Table 2, Figure S1). The mean number of teeth at baseline in the IPT and MPT groups was 22.6 (SD = 3.8) and 22.0 (SD = 4.2) ($P = .611$), respectively, with a similar number of tooth extractions performed in both groups (0.7 [SD = 1.5] and 0.2 [SD = 0.5], respectively [$P = .130$]).

Compared with the MPT group, the IPT group had lower values for PII at 6 months (absolute difference, 0.4; 95% CI: [0.1; 0.7]; $P = .003$). Six months after therapy, patients in the IPT group had lower scores for BOP (absolute difference, 31.1%; 95% CI: [22.3; 40.0]; $P < .001$) than those in the MPT group. Similarly, 6 months after therapy, patients in the IPT group had significantly less sites with PPD ≥ 4 mm (32.6%; 95% CI: [23; 42]; $P < .001$) and experienced a significantly higher CAL gain than the control group (1.0, SD = 0.8 mm vs. 0.3, SD = 0.6 mm, respectively [$P < .001$]).

The microbiological outcomes, expressed as counts and proportions of each target bacterial species at each time point, are presented in Tables S3 and S4. At baseline, both the test group and the control group harboured similar proportions of periodontal pathogens (*P. gingivalis* in 15.6% and 20.5%, respectively, and *P. intermedia* in 5.5% and 6.3%, respectively). Total anaerobic counts were not significantly different between both groups throughout the study, although significant reductions occurred in the IPT group 3 and 6 months post-treatment. Similarly, the counts of *P. gingivalis* were significantly lower in the IPT group, to the MPT group, at 3 (6.9 ln CFUs; 95% CI: [4.0; 9.9]; $P < .001$) and 6 months (6.6 ln CFUs; 95% CI: [3.5; 9.6]; $P < .001$). Significantly reduced counts were also found for *P. intermedia* (5.7 in CFUs; 95% CI: [2.9; 8.5]; $P < .001$; and 3.1 in CFUs; 95% CI: [0.3; 6.0]; $P = .031$) and *T. forsythia* (5.7 in CFUs; 95% CI: [3.2; 8.2]; $P < .001$and 3.1 in CFUs; 95% CI: [0.2; 6.0]; $P = .035$) in the IPT group at 3 and 6 months post-therapy.

TABLE 1 Baseline characteristics of the intention-to-treat population with metabolic syndrome (MetS)

Variable[a]	Intensive periodontal therapy (n = 32)	Minimal periodontal therapy (n = 31)	*P* value
Age, years	56.7 (6.5)	58.3 (5.8)	.319
Sex			.839
Male, %	22 (68.8%)	22 (70.9%)	
Female, %	10 (31.2%)	9 (29.1%)	
Smoking status			.112
Never smoker, %	15 (46.9%)	11 (35.5%)	
Former smoker, %	9 (28.1%)	17 (54.8%)	
Current smoker, %	8 (25.0%)	3 (9.7%)	
BMI, kg/m^2	39.1 (5.6)	38.0 (4.7)	.618
WC, cm	120.1 (18.4)	119.0 (9.1)	.848
BP, mmHg			
Systolic BP, mmHg	148.1 (21.5)	138.6 (18.3)	.112
Diastolic BP, mmHg	91.3 (18.2)	84.1 (11.2)	.143
CRP, mg/L	3.9 (2.9) 2.8 (IQR 1.5-4.9)	3.9 (3.4) 2.4 (IQR 1.4-4.9)	.831
Subjects with CRP levels ≥3 mg/L, %	17 (53.1%)	17 (54.8%)	.975
α-1 antitrypsin, mg/dL	145.6 (29.7)	138.5 (28.1)	.369
Fibrinogen, mg/dL	419.7 (108.7)	398.5 (89.1)	.259
WBC, K/μL	7.8 (1.9)	7.5 (1.7)	.445
Inflammatory mediators in serum, pg/mL			
IL-1β	1.5 (0.9)	1.9 (1.2)	.161
IL-6	2.2 (1.8) 1.9 (IQR 1.3-2.3)	2.8 (1.9) 2.8 (IQR 1.4-3.9)	.178
IL-8	6.9 (9.7) 3.3 (IQR 2.3-5.7)	5.4 (3.0) 4.7 (IQR 3.5-6.3)	.105
TNF-α	7.9 (6.2) 5.7 (IQR 4.8-10.2)	8.7 (8.6) 6.3 (IQR 4.1-8.9)	.957
HbA1c, %	6.3 (1.2) 6.1 (IQR 5.5-6.9)	6.0 (1.0) 5.7 (IQR 5.2-6.5)	.328
Subjects with HbA1c ≥ 7%, %	10 (31.25%)	8 (25.8%)	.774
FPG, mg/dL	128.6 (30.3) 125 (IQR 107-154)	133.0 (51.7) 112 (IQR 97-159)	.500
Fasting insulin, mIU/L	19.3 (10.8) 18.7 (IQR 12.3-27.6)	14.5 (9.3) 11.1 (IQR 9.4-15.8)	.033
HOMA2-β cell function	104.8 (69.0)	92.7 (50.6)	.519
HOMA2-insulin sensitivity	59.0 (55.3) 38.3 (IQR 30.0-62.3)	62.6 (28.0) 66.5 (IQR 43.3-75.1)	.061
HOMA2-insulin resistance	2.6 (1.4) 2.6 (IQR 1.6-3.3)	2.0 (1.2) 1.5 (IQR 1.3-2.3)	.063
Total cholesterol, mg/dL	174.8 (34.7)	189.4 (48.4)	.203
HDL cholesterol, mg/dL	46.1 (13.3)	46.9 (12.4)	.858
LDL cholesterol, mg/dL	114.3 (34.7)	105.7 (44.9)	.535
TG, mg/dL	129.5 (52.3)	136.6 (42.5)	.582
Creatinine, mg/dL	0.9 (0.5) 0.8 (IQR 0.7-0.9)	0.9 (0.3) 0.9 (IQR 0.8-1.0)	.461

Abbreviations: BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; FPG, fasting plasma glucose; HOMA2, homeostasis model assessment 2; TG, triglycerides; WBC, white blood cell count; WC, waist circumference.

[a]Data are mean (standard deviation [SD]), n (%), or median (interquartile range [IQR]).

TABLE 2 Periodontal variables in both groups over time

Variable	Baseline (BL)		3 months (3 M)		ΔBL-3 M		6 months (6 M)		ΔBL-6 M	
	Mean (SD)	*P* value	Mean (SD)	*P* value	Mean (SD)	*P* value	Mean (SD)	*P* value	Mean (SD)	*P* value
Plaque index (0-3)										
IPT group	1.8 (0.4)	.444	0.8 (0.3)*	.002	1.0 (0.5)	.012	0.8 (0.3)*	.003	1.0 (0.4)	.013
MPT group	1.9 (0.5)		1.3 (0.7)*		0.6 (0.6)		1.2 (0.6)*		0.7 (0.5)	
Gingival index (0-3)										
IPT group	1.8 (0.4)	.091	0.6 (0.2)*	<.001	1.2 (0.5)	<.001	0.6 (0.3)*	<.001	1.1 (0.5)	<.001
MPT group	2.0 (0.4)		1.4 (0.7)*		0.6 (0.6)		1.4 (0.6)*		0.5 (0.5)	
Whole mouth bleeding score (%)										
IPT group	59.8% (20)	.139	24.9% (17)*	<.001	32.8% (21)	.025	20.5% (11)*	<.001	37.6% (17)	<.001
MPT group	67.8% (20)		48.4% (21)*		19.4% (20)		51.6% (18)		16.1% (21)	
Mean periodontal pocket depth (mm)										
IPT group	3.7 (0.9)	.747	2.7 (0.3)*	<.001	1.1 (0.6)	<.001	2.6 (0.3)*	<.001	1.2 (0.6)	<.001
MPT group	3.8 (0.9)		3.5 (0.7)		0.3 (0.8)		3.5 (0.6)		0.3 (0.8)	
Percentage of periodontal pockets ≥4 mm										
IPT group	55.3% (22)	.427	13.9% (12)*	<.001	39.4% (20)	<.001	11.2% (10)*	<.001	43.7% (20)	<.001
MPT group	59.7% (19)		41.2% (23)*		18.5% (18)		43.9% (21)*		15.9% (19)	
Percentage of periodontal pockets >6 mm										
IPT group	3.5% (6)	.739	0.1% (0)*	.013	4.0% (6)	.007	0.3% (1)*	.016	3.8% (6)	.018
MPT group	4.1% (7)		3.6% (6)		0.6% (2)		3.3% (6)		0.8% (2)	
Mean CAL, mm										
IPT group	4.9 (1.0)	.293	3.9 (1.0)*	.010	0.9 (0.7)	.001	3.8 (0.9)*	.001	1.0 (0.8)	<.001
MPT group	5.2 (1.3)		4.8 (1.4)		0.3 (0.5)		4.9 (1.3)		0.3 (0.6)	
Number of teeth										
IPT group	22.6 (3.8)	.611	21.9 (4.1)	.944	0.7 (1.5)	.130	21.9 (4.1)	.944	0.7 (1.5)	.130
MPT group	22.0 (4.2)		21.9 (4.2)		0.2 (0.5)		21.9 (4.2)		0.2 (0.5)	

Abbreviations: CAL, clinical attachment level; IPT, intense periodontal therapy; MPT, minimal periodontal therapy; SD, standard deviation.
*Intra-group significant difference when compared with baseline ($P < .05$).

3.3 | Impact of the interventions on systemic inflammatory status

The primary outcome, mean hs-CRP concentration, was reduced after 3 and 6 months in the patients in the test group (IPT), but not in the control group (MPT) (Figure 2). The difference between treatment groups (adjusted for age, sex, smoking status, BMI and baseline hs-CRP) was 1.4 mg/L (95% CI [0.5; 2.2]; $P = .001$) at 3 months and 1.2 mg/L (95% CI [0.4; 2.0]; $P = .004$) at 6 months (Table 3). If assigned to the IPT group versus the MPT group, the odds ratio for a patient to move from a hs-CRP value of ≥3 to <3 mg/L was 5.4 (95% CI [1.0; 31.6]; $P = .040$). At 6 months, the percentage of patients in the IPT group experiencing a reduction in hs-CRP levels was 68.8%, while this percentage was 29.0% in the MPT group ($P < .001$). Among the variables included in the multilevel linear regression, only belonging to the IPT group ($P = .004$), baseline hs-CRP levels ($P < .001$) and being a smoker ($P = .014$) significantly and independently predicted the variance of hs-CRP decline over 6 months.

In the secondary outcomes, significant reductions in IL-1β and TNF-α at 3 months were observed in the IPT group compared with the MPT group (Table 3). However, no differences between the groups were observed for these biomarkers at 6 months, or for the other inflammatory markers (α-1 antitrypsin, fibrinogen, WBC count, IL-6 and IL-8) at any time point after therapy. No differences in BMI or WC between patients in the IPT or MPT groups were observed throughout the study.

3.4 | Metabolic, vascular and renal outcomes

After 3 months, HbA1c was reduced in patients in the IPT group compared with the MPT group (Table 4, Figure S2), with a difference, after adjustment for covariates, of 0.3% (95% CI: [0.1; 0.6]; $P = .013$). Post hoc analyses showed that the proportion of patients with an HbA1c of ≥7% decreased significantly in the IPT group, from 31.25% at baseline to 18.8% at 3 months ($P = .028$), with no changes in the MPT

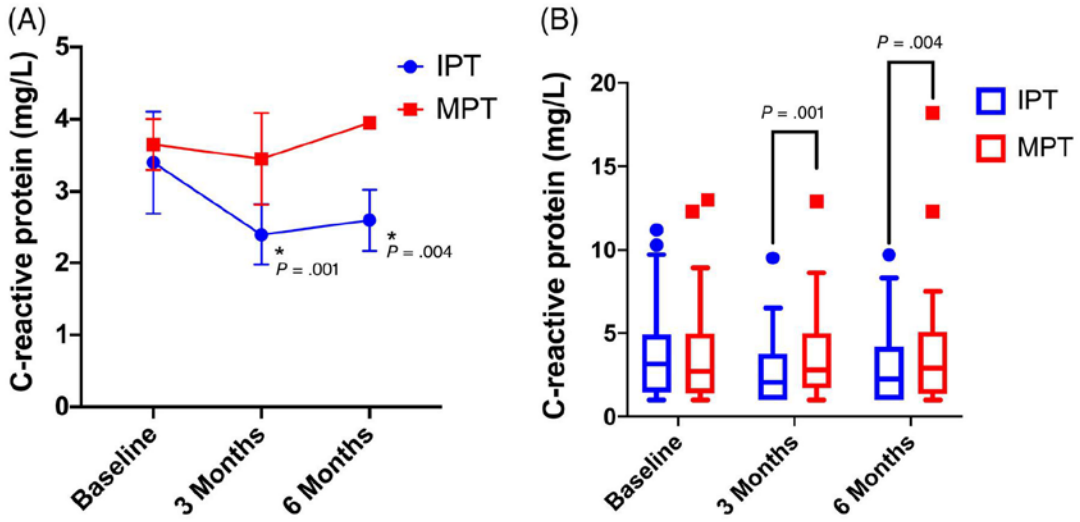

FIGURE 2 C-reactive protein (CRP) during the 6-month study period. (A) Data are unadjusted means and standard deviations (SDs). Adjusted *P*-values (for age, sex, smoking, baseline values and body mass index) for between-group difference at 3 and 6 months were $P = .001$ and $P = .004$, respectively. (B) Boxplot showing CRP concentrations over visits by study group. Values are presented as median, 25% and 75% quantiles, whiskers corresponding to minimum and maximum values within the interval (25% quantile − 1.5 × interquartile range, 75% + 1.5 × interquartile range), and outliers. IPT, intense periodontal therapy; MPT, minimal periodontal therapy

group. No differences between both groups were observed at 6 months. FPG, fasting insulin concentrations, HOMA2 scores and lipid fractions did not significantly differ between groups or visits. The multilevel linear regression determined that the variance in HbA1c, observed at 3 months, was only significantly predicted by being in the IPT group ($P = .013$) and by the baseline HbA1 percentage ($P < .001$), without any significant additional effect in the model for age, sex, BMI or smoking status.

Systolic blood pressure was significantly reduced at 3 months in the IPT group compared with the MPT group, after adjustment for covariates (7.3 mmHg; 95% CI: [1.9; 12.6]; $P = .008$; Table 4, Figure S1). The reduction for diastolic blood pressure in the IPT group compared with the MPT group lasted for 6 months (at 3 months, 7.8 mmHg; 95% CI: [1.3; 14.4]; $P = .019$; at 6 months, 11.0 mmHg; 95% CI 2.9, 19.1; $P = .009$). No differences in creatinine between patients in the IPT or MPT groups were observed throughout the study.

4 | DISCUSSION

The present study has shown that effective periodontal treatment in patients with severe periodontitis and MetS results in significant reductions of systemic inflammation and atherosclerotic risk biomarkers. The tested periodontal treatment, consisting of non-surgical subgingival instrumentation with adjunctive systemic antibiotic administration (azithromycin 500 mg q.d. for 3 days), compared with a control group with community periodontal care (supragingival plaque control), resulted in statistically significant reductions of hs-CRP, IL-1β and TNF-α at 3 months, and hs-CRP at 6 months. Furthermore, significant improvements in vascular function and significant reductions in HbA1c also occurred in the test group.

The impact of this tested periodontal intervention on hs-CRP was a 30.8% reduction from baseline values and a difference of 1.2 mg/L at 6 months compared with the control treatment. These results show that the improvements in periodontal health in these MetS patients, despite actively following strict cardiovascular risk reduction protocols (including specific cardioprotective drug therapies), significantly improved their hs-CRP levels, and hence their cardiovascular risk, as it has recently been reported that hs-CRP reductions significantly reduce cardiovascular events, cardiovascular mortality and all-cause mortality.[25]

Because MetS is a cluster of medical conditions associated with cardiovascular risk, these results may, therefore, apply to other conditions with increased cardiovascular risk. Indeed, clinical trials and systematic reviews with meta-analyses, evaluating the impact of periodontal treatment in CVD patients undergoing secondary prevention, have shown similar outcomes.[26–29] Bokhari et al., assessing the impact of periodontal treatment in patients with coronary heart disease (CHD) and periodontitis, reported a 30% decrease in mean hs-CRP levels.[27] By contrast, the results from the Periodontitis and Vascular Events (PAVE) study[30] carried out in CHD patients with periodontitis reported that periodontal therapy was not able to maintain the reduction of serum hs-CRP levels at 6 months. However, this study population did not have severe periodontitis and the periodontal therapy rendered was not able to show a significant effect on the periodontal variables compared with the control group. In the present investigation, all patients had severe periodontitis, and there was a

TABLE 3 Inflammatory biomarkers and clinical variables 3 and 6 months after periodontal treatment, by study group

Variable	3 months (3M)			6 months (6M)		
	Mean (SE) or n (%)	Δ3M (95% CI)	*P* value	Mean (SE) or n (%)	Δ6M (95% CI)	*P* value
CRP, mg/L						
IPT group	2.7 (0.4)*	1.4 (0.5, 2.2)	.001	2.9 (0.4)*	1.2 (0.4, 2.0)	.004
MPT group	3.9 (0.6)			4.0 (0.8)		
Subjects with CRP levels ≥3 mg/L, %						
IPT group	13 (40.6%)*	5.4 (1.0, 31.6)	.040	14 (43.8%)*	2.6 (0.4, 15.9)	.312
MPT group	18 (58.1%)			18 (58.1%)		
α-1 antitrypsin, mg/dL						
IPT group	138.4 (6.2)	−4.9 (−15.6, 5.8)	.368	137.5 (5.7)	−6.9 (−20.3, 6.4)	.308
MPT group	130.0 (5.0))			127.6 (5.2)		
Fibrinogen, mg/dL						
IPT group	421.8 (20.4)	−27.4 (−64.3, 9.4)	.144	419.6 (21.8)	−25.0 (−66.6, 16.5)	.238
MPT group	398.3 (17.9)			400.5 (16.1)		
WBC, K/μL						
IPT group	7.5 (0.4)	−0.1 (−0.7, 0.5)	.768	7.9 (0.7)	−0.4 (−1.5, 0.7)	.515
MPT group	7.8 (0.3)			7.6 (0.2)		
Serum IL-1β, pg/mL						
IPT group	0.9 (0.1)*	1.2 (0.0, 2.4)	.046	1.5 (0.2)	−0.1 (−0.5, 0.3)	.601
MPT group	2.3 (0.5)			1.5 (0.2)		
Serum IL-6, pg/mL						
IPT group	1.9 (0.4)	0.3 (−0.8, 1.3)	.584	2.0 (0.4)	0.1 (−0.9, 1.2)	.806
MPT group	2.6 (0.4)			2.5 (0.4)		
Serum IL-8, pg/mL						
IPT group	4.6 (1.1)*	1.0 (−0.9, 2.8)	.307	5.0 (1.2)	0.8 (−2.0, 3.5)	.584
MPT group	5.4 (0.8)			6.0 (1.2)		
Serum TNF-α, pg/mL						
IPT group	6.4 (0.8)*	3.2 (0.2, 6.2)	.037	6.3 (0.8)*	1.6 (−1.6, 4.8)	.333
MPT group	10.0 (2.3)			8.2 (1.4)		
BMI, kg/m^2						
IPT group	39.1 (1.6)	0.1 (−0.2, 0.5)	.503	39.2 (1.6)	0.0 (−0.1, 0.2)	.611
MPT group	38.0 (1.6)			38.0 (1.6)		
WC, cm						
IPT group	120.1 (4.5)	0.2 (−0.5, 1.0)	.527	120.1 (4.6)	0.4 (−0.1, 1.0)	.115
MPT group	119.2 (2.7)			119.2 (2.7)		

Abbreviations: BMI, body mass index; CRP, C-reactive protein; IL, interleukin; IPT, intense periodontal therapy; MPT, minimal periodontal therapy; SIS, Summary Inflammatory Score; TNF, tumor necrosis factor; WBC, white blood cell count; WC, waist circumference.

Note: Data are unadjusted mean and standard error (SE). *P* values are for the adjusted Δ values (Δ3M and Δ6M), that are adjusted for baseline values, age, sex, smoking habit and BMI. In case of the percentage of subjects with CRP levels ≥3 mg/L, the adjusted Δ values are expressed as odds ratios and 95% confidence intervals (CIs). Adjustment for BMI was made for baseline values, age, sex and smoking habit.

*Intra-group significant difference when compared with baseline ($P < .05$).

clear and significant improvement in all periodontal outcomes in the test group compared with the control group.

Despite periodontitis being defined as a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms,[19] most of the previous reports evaluating the impact of periodontal treatment on cardiovascular risk have relied on surrogate markers of exposure that were either clinical (probing depths) or radiographic (bone loss), but they did not assess exposure by measuring the bacterial burden or the presence of putative pathogens.[16,27,31] In the present investigation we have identified high counts of anaerobic bacteria and high proportions and counts of *P. gingivalis*, a keystone periodontal pathogen strongly associated with periodontitis, in all

TABLE 4 Metabolic, vascular and renal measures at 3 and 6 months after periodontal treatment by study group

Variable	3 months (3M)			6 months (6M)		
	Mean (SE) or n (%)	Δ3M (95% CI)	*P* value	Mean (SE) or n (%)	Δ6M (95% CI)	*P* value
HbA1c, %						
IPT group	5.9 (0.1)*	0.3 (0.1, 0.6)	.013	6.0 (0.1)*	0.2 (0.0, 0.5)	.110
MPT group	6.1 (0.2)			6.1 (0.2)		
Subjects with HbA1c ≥ 7%, %						
IPT group	6 (18.8%)*	21.7 (0.8-623.3)	.073	9 (28.1%)	0.7 (0.1-4.9)	.739
MPT group	8 (25.8%)			7 (22.6%)		
FPG, mg/dL						
IPT group	123.3 (7.9)	12.8 (−3.0, 28.6)	.112	121.0 (6.3)	8.9 (−9.2, 26.9)	.336
MPT group	130.0 (8.8)			130.5 (9.7)		
Fasting insulin, mIU/L						
IPT group	17.2 (2.9)	−1.4 (−6.5, 3.6)	.583	14.3 (2.1)	1.3 (−3.0, 5.7)	.548
MPT group	14.1 (1.4)			14.4 (1.7)		
HOMA2-β cell function						
IPT group	106.0 (14.8)	−13.6 (−37.2, 10.1)	.260	106.1 (14.0)	2.2 (−21.6, 26.0)	.856
MPT group	87.2 (10.6)			100.4 (11.9)		
HOMA2-insulin sensitivity						
IPT group	67.0 (13.6)	−6.1 (−27.3, 15.1)	.573	65.8 (11.6)	−8.2 (−24.7, 8.3)	.330
MPT group	57.9 (5.4)			59.7 (5.3)		
HOMA2-insulin resistance						
IPT group	2.3 (0.4)	−0.2 (−0.8, 0.5)	.666	2.2 (0.3)	0.0 (−0.6. 0.6)	.940
MPT group	2.0 (0.2)			2.0 (0.2)		
Total cholesterol, mg/dL						
IPT group	184.0 (8.4)	−9.7 (−20.9, 1.5)	.090	183.5 (7.5)	−5.2 (−17.3, 6.9)	.397
MPT group	180.6 (8.1)			189.9 (9.2)		
HDL cholesterol, mg/dL						
IPT group	46.2 (3.8)	0.8 (−8.1, 9.7)	.858	47.2 (2.7)	−4.0 (−8.8, 0.7)	.097
MPT group	47.1 (3.1)			48.4 (2.7)		
LDL cholesterol, mg/dL						
IPT group	109.6 (8.5)	−5.7 (−16.9, 5.6)	.327	107.6 (6.6)	−2.2 (−17.9, 13.4)	.779
MPT group	103.5 (7.0)			107.5 (8.3)		
TG, mg/dL						
IPT group	136.5 (9.7)	−0.2 (−18.5, 18.1)	.984	125.6 (9.7)	1.2 (−16.5, 18.9)	.895
MPT group	155.4 (17.5)			131.7 (8.3)		
Systolic blood pressure, mmHg						
IPT group	136.4 (3.0)*	7.3 (1.9, 12.6)	.008	136.4 (2.8)	4.7 (−11.7, 21.1)	.574
MPT group	139.4 (2.7)			144.4 (4.1)		
Diastolic blood pressure, mmHg						
IPT group	84.8 (3.8)	7.8 (1.3, 14.4)	.019	81.8 (2.8)	11.0 (2.8, 19.1)	.009
MPT group	89.6 (5.4)			86.9 (1.8)		
Creatinine, mg/dL						
IPT group	1.0 (0.1)	0.0 (0.0, 0.1)	.353	1.0 (0.1)	0.0 (−0.1, 0.1)	.924
MPT group	0.9 (0.1)			1.0 (0.1)		

Abbreviations: FPG, fasting plasma glucose; HDL, high-density lipoproteins; IPT, intense periodontal therapy; LDL, low-density lipoproteins; MetS, metabolic syndrome; MPT, minimal periodontal therapy; TG, triglycerides.

Note: Data are unadjusted mean and standard error (SE). *P* values are for the adjusted Δ values (Δ3M and Δ6M), that are adjusted for baseline values, age, sex, smoking habit and body mass index. In case of the proportions (%), the adjusted Δ values are expressed as odds ratios and 95% confidence intervals (CI).

*Intra-group significant difference when compared with baseline ($P < .05$).

patients at baseline. The tested intervention significantly reduced both the counts of anaerobic bacteria and *P. gingivalis*. Although previous cross-sectional studies have shown an independent significant association between presence of a significant subgingival bacterial burden and levels of periodontal pathogens with surrogate measurements of atherosclerotic risk as intima-media thickness or hs-CRP,[32] this is the first RCT to show that effective periodontal treatment significantly reduced these microbiological exposure measurements and that this microbiological impact was associated with significant reductions in hs-CRP.

Other significant improvements associated with the effective periodontal treatment were the significant reduction (0.3%) in HbA1c at 3 months, and a significant reduction in arterial blood pressure, thus suggesting that this effect was not limited to the improvement in systemic inflammation, but also impacted vascular function and metabolic control. In fact, periodontal inflammation has been associated with both insulin resistance and endothelial dysfunction.[28,31,33] The fact that the significance in HbA1c reduction in the treatment group was lost at 6 months may be caused by the lack of repeated periodontal interventions during the course of the study. Effective periodontal maintenance care has been shown in other studies to maintain a positive effect in glycaemic control.[20,29]

Similarly, recent studies have shown a significant association between periodontitis and hypertension and have suggested that periodontal treatment could reduce arterial blood pressure.[34] In this RCT, we have reported an adjusted statistically significant reduction in both systolic and diastolic blood pressure (7.8 and 7.3 mmHg, respectively), 3 months after effective periodontal treatment. These findings may be meaningful, because a 10 mmHg reduction in systolic blood pressure or a 5 mmHg reduction in diastolic blood pressure has been associated with a 25%-30% reduction of cardiovascular events,[35] and half of hypertensive adults remain poorly controlled despite effective medications.[36]

The current clinical trial, however, may have relevant limitations. One is that the tested intervention consisted of a combination of subgingival debridement and the adjunctive administration of azithromycin. This antibiotic has shown significant reductions in a global inflammatory score consisting of hs-CRP, IL-1β, IL-6 and TNF-α at 6 months, but not on CRP alone,[37] and hence the cardiovascular effect shown in this study could be attributed to the antibiotic rather than the periodontal therapy. However, the adjunctive use of systemic antimicrobials to subgingival instrumentation is clearly justified by the periodontitis severity in the selected sample, as this modality of therapy has shown a significant added effect on clinical and microbiological outcomes compared with subgingival debridement alone in severe periodontitis patients,[38] as well as in RCTs evaluating the impact of intensive periodontal therapy in cardiovascular risk outcomes.[29,31]

Another possible limitation could be the selection of the main outcome variable (hs-CRP) as a surrogate for cardiovascular risk, as its predictive value may be limited,[39] although this is the biomarker most frequently reported in screening and cardiovascular risk reclassification.[40,41] Further investigations should also focus on other surrogate measures of subclinical atherosclerosis, such as coronary artery calcium or, when possible, on true endpoints, such as major cardiovascular events.

In conclusion, the results of the present study provide evidence that effective periodontal therapy reduced the cardiovascular risk in patients with MetS and severe periodontitis by significantly reducing hs-CRP levels, proinflammatory mediators, blood pressure and HbA1c levels. Larger studies with a longer follow-up are needed to determine whether these shown short-term benefits can be sustained over the long term and result in reductions in the cardiovascular morbidity and mortality in this patient population.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors contributed significantly to this work. MS, DH and AZ conceived the study. EM, ML, HV, MM, JM and LV contributed to data collection. Statistical analysis was carried out by EM. EM drafted the manuscript. All authors critically revised the manuscript and approved the final version.

PEER REVIEW

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ORCID

Eduardo Montero https://orcid.org/0000-0003-2525-8529

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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STUDY #4

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Development and validation of a predictive model for periodontitis using NHANES 2011-2012 data

Aim: To develop and validate a predictive model for moderate-to-severe periodontitis in the adult USA population, with data from the 2011–2012 National Health and Nutrition Examination Survey (NHANES) cycle.

Material and Methods: A subset of 3017 subjects aged >30 years, with >14 teeth present and having received a periodontal examination in addition to data collected on cardio-metabolic risk measures (smoking habit, body mass index [BMI], blood pressure, total cholesterol and glycated hemoglobin [HbA1c]) were used for model development by multivariable logistic regression.

Results: The prevalence of moderate and severe periodontitis using CDC/AAP classification was 37.1% and 13.2%, respectively. A multivariable logistic regression model revealed that HbA1c ≥5.7% was significantly associated with moderate-to-severe periodontitis (odds ratio, OR = 1.29; $p < 0.01$). A predictive model including age, gender, ethnicity, HbA1c and smoking habit as variables had 70.0% sensitivity and 67.6% specificity in detecting moderate-to-severe periodontitis in US adults.

Conclusions: Periodontitis is a common disease in North American adults, and its prevalence is significantly higher in individuals with pre-diabetes or diabetes. The present study demonstrates that a model including age, gender, ethnicity, HbA1c and smoking habit could be used as a reliable screening tool for periodontitis in primary medical care settings to facilitate referral of patients at risk for periodontal examination and diagnosis.

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Development and validation of a predictive model for periodontitis using NHANES 2011–2012 data

Eduardo Montero[1] | David Herrera[1] | Mariano Sanz[1] | Sangeeta Dhir[2] | Thomas Van Dyke[3,4] | Corneliu Sima[3,4]

[1]ETEP (Etiology and Therapy of Periodontal Diseases) Research Group, University Complutense, Madrid, Spain

[2]Department of Dentistry, Consultant Periodontist, Max Super Specialty Hospital, Saket, New Delhi, India

[3]Center for Clinical and Translational Research, Forsyth Institute, Cambridge, Massachusetts

[4]Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts

Correspondence
Eduardo Montero, Faculty of Odontology, Section of Graduate Periodontology, University Complutense of Madrid. Madrid, Spain.
Email: eduardomonterosolis@ucm.es
Corneliu Sima, Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, MA.
Email: corneliu_sima@hsdm.harvard.edu

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Abstract

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KEYWORDS
diabetes, endocrinology, glycated haemoglobin, HbA1c, periodontitis, predictive modelling

1 | INTRODUCTION

Periodontitis is a chronic inflammatory disease associated with oral biofilm dysbiosis and unresolved inflammation leading to destruction of tooth supporting structures. Severe periodontitis is estimated to affect 11% of world population what implies a significant deterioration of oral health-related quality of life (OHrQL) (Cunha-Cruz, Hujoel, & Kressin, 2007; Gerritsen, Allen, Witter, Bronkhorst, & Creugers, 2010) and heavy economic burdens on healthcare systems (Kassebaum et al., 2014).

While the role of genetics has been associated with up to 50% of susceptibility to periodontitis, there is ample evidence on the impact of modifiable risk factors. In fact, poor oral hygiene, smoking and uncontrolled diabetes increase the odds of developing periodontitis up to 5-fold (Michalowicz et al., 2000; Borgnakke, Ylostalo, Taylor, & Genco, 2013; Chapple & Genco, 2013). Hyperglycaemia is known

to favour pro-inflammatory priming of periodontal tissues increasing the risk for gingivitis in patients with diabetes (Salvi, Kandylaki, Troendle, Persson, & Lang, 2005; Sima, Rhourida, Van Dyke, & Gyurko, 2010). Epidemiological evidence also demonstrated that poor control of glycaemia correlated with higher prevalence, severity and progression rate of periodontitis compared to normo-glycemic individuals (Borgnakke et al., 2013).

Severe periodontitis contributes to the systemic inflammatory burden, hence affecting the overall health, and may impact other chronic diseases such as diabetes mellitus and atherosclerotic cardiovascular diseases (Tonetti, 2009; Chapple & Genco, 2013; Tonetti & Van Dyke, 2013). This relationship between diabetes and periodontitis is clearly bidirectional, since significant improvements in glycemic control, measured by the percentage of glycated haemoglobin (HbA1c) have been observed after periodontal therapy (Chapple & Genco, 2013). Central to these associations seems to be the unresolved systemic inflammation indicated by high-sensitivity C-reactive protein measurements and white blood cell counts (Genco & Van Dyke, 2010; Demmer et al., 2013).

Frequently associated with both diabetes and periodontitis are overweight and obesity, conditions affecting around 35% of US adults (Flegal, Carroll, Kit, & Ogden, 2012). Numerous studies have reported a positive association between body mass index (BMI) ≥25 and periodontitis, although the magnitude of this association has varied in different populations (Suvan, D'Aiuto, Moles, Petrie, & Donos, 2011; Suvan et al., 2015). The odds of having periodontitis, adjusted for age, gender, smoking, alcohol consumption and frequency of tooth brushing, seem to increase with BMI in different populations (Suvan et al., 2011). Similarly, there has been a positive association between the metabolic syndrome (increased blood pressure, elevated plasma glucose, excess body fat around the waist and abdominal area and altered cholesterol levels) and periodontitis (Shimazaki et al., 2007; Saxlin et al., 2008; Nesbitt et al., 2010; Gomes-Filho et al., 2016). In the third NHANES survey, individuals ≥45 years of age suffering from severe periodontitis were 2.3 times (95% confidence interval [CI]: 1.13–4.47) more likely to have metabolic syndrome compared with unaffected individuals (D'Aiuto et al., 2008). Unresolved inflammation is the most plausible biological explanation for these associations. Therefore, reinforcement of preventive strategies aimed at reducing periodontitis-associated burden through integrative approaches to pro-inflammatory conditions including obesity, pre-diabetes and diabetes is necessary.

The aim of this study was to assess the associations between cardio-metabolic risk measures and moderate-to-severe periodontitis using the National Health and Nutrition Examination Survey 2011–2012 data set (NHANES 2011-2012), which is a sample representative of US non-institutionalized adult population. A predictive model using a combination of cardio-metabolic and socio-demographic variables was created and validated for predicting moderate-to-severe periodontitis to be used as screening tool by physicians in primary care settings.

Clinical Relevance

Scientific rationale for study: Periodontitis has been associated with several cardio-metabolic risk factors, diabetes and cardiovascular disease. A model comprising commonly registered risk factors for these diseases would be useful for their co-management by primary care physicians and periodontists, to control the associated systemic inflammatory burden.

Principal findings: A predictive model including age, gender, ethnicity, HbA1c and smoking habit as variables presented appropriate sensitivity and specificity to be used as screening tool for moderate-to-severe periodontitis in primary medical care settings. In any case, the absence of risk factors/determinants included in the model should be interpreted as an indicator of periodontal health.

Practical implications: The results from this study support the concept of integrative approaches by physicians and periodontists in the management of cardio-metabolic disorders and periodontitis. The predictive model presented may be used by physicians to integrate oral screening in the patient management workflow and reinforces the need for guidelines to screen for periodontitis in primary medical care settings. This will further facilitate inter-disciplinary co-management of pre-diabetes/diabetes and periodontitis.

2 | MATERIAL AND METHODS

2.1 | Study design and sample

NHANES 2011–2012 was a cross-sectional study conducted by the National Center for Oral Health Statistics (NCHS) which is part of the Center for Disease Control and Prevention. NHANES 2011–2012 was designed to evaluate the health and nutritional status of adults and children in the United States using a multistage, stratified, clustered probability sample of the US civilian, non-institutionalized population ≥2 years old. The protocols for NHANES 2011–2012 were approved by the institutional review board of the NCHS. Informed consent was obtained from all participants (Johnson, Dohrmann, Burt, & Mohadjer, 2014).

Among the 9,756 subjects evaluated in NHANES 2011–2012, the present study has focused on a subset of participants aged >30 years with the following registered data: age, gender, ethnicity, smoking habit, BMI, blood pressure, total cholesterol and HbA1c. Among these stratified samples, 3,017 subjects were identified as having >14 teeth and having received a periodontal examination. This study conforms with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting cross-sectional studies. Moreover, this manuscript also conforms with the TRIPOD (Transparent Reporting of a Multivariable Prediction Model

for Individual Prognosis or Diagnosis) guidelines for reporting predictive models (Moons et al., 2015). Additional information on the studied sample can be accessed at Supplemental Methods.

2.2 | Clinical periodontal outcomes

Clinical attachment level (CAL) and probing pocket depth (PPD) measurements were recorded at 6 sites/tooth. CAL was measured from the cement–enamel junction to the base of the sulcus/pocket as a composite measurement of recession (distance from free gingival margin to cement–enamel junction) and PPD (distance from free gingival margin to the base of the sulcus).

Subjects were categorized in one of the three case definitions reported by Page and Eke (Page & Eke, 2007) for their use in population-based studies:

- Severe periodontitis, if the patient presented ≥2 inter-proximal sites with CAL ≥6 mm (not on the same tooth) and ≥1 inter-proximal site with PPD ≥5 mm.
- Moderate periodontitis, if the patient presented ≥2 inter-proximal sites with CAL≥4 mm (not on the same tooth) or ≥2 inter-proximal sites with PPD ≥5 mm.
- No or mild periodontitis, neither "moderate" nor "severe" periodontitis.

Moderate and severe periodontitis were grouped into the same category, moderate-to-severe periodontitis, for the statistical analysis.

2.3 | Data analysis

All data analyses were performed with a software package (STATA v.13 with SVY package, StataCorp, College Station, TX, USA), which accounts and weights the multistage stratified, clustered sampling method of NHANES III. Means and standard deviations (SDs) were used to describe the demographic, cardio-metabolic and periodontal characteristics of participants.

Candidate predictors were categorized in order to facilitate clinicians' use of the prediction model. Educational level was categorized into: (a) secondary school, (b) high school graduate, (c) college degree and (d) college graduate or above. Annual household income was presented as follows: (a) <20,000$, (b) 20,000-100,000$ and (c) >100,000$. The definition of smoking was based on participants' answers to the following questions: (a) "Have you smoked >100 cigarettes in life?" and (b) "Do you smoke cigarettes now?". If participants answered "no" to both questions, they were coded as non-smokers; if participants answered "yes" to question (a) but "no" to question (b) they were coded as past smokers; if participants answered "yes" to both questions, they were coded as current smokers. Glucose regulation was classified as normal (HbA1<5.7%) or abnormal (HbA1c ≥5.7%). Usual categories for BMI were employed (underweight–normal weight/overweight/obese). Blood pressure and total cholesterol values were dichotomized on the basis of the thresholds established for high blood pressure and hypercholesterolaemia.

Odds ratios (ORs), along with the associated 95% CI, for both periodontal status (either no/mild periodontitis or moderate/severe periodontitis) and mean CAL (after transforming it into a categorical variable; 0–3 mm, 4–6 mm, >6 mm) were estimated separately for each potential risk indicator using multivariate logistic regression with adjustment for potential confounding factors such as age, gender, ethnicity, smoking habit, educational level or annual household income. In the case of mean CAL ordinal logistic regression analysis was used as a consequence of the hierarchical relation between categories.

For the predictive model, first-order interaction terms were evaluated through a global signification test (chunk test). As long as the result was non-significant, all interactions were excluded from the candidate model. Candidate socio-demographic and cardio-metabolic measures were included in a multivariable logistic regression analysis following a backward elimination approach for removal of variables. The best model was selected using the "all possible equations" strategy and both the area under the curve (AUC) and the Akaike's information criterion (AIC) criteria. Use of the AIC for selection has been considered as an attractive option, as it accounts for model fit while penalizing for the number of parameters being estimated (Sauerbrei, Boulesteix, & Binder, 2011). Candidate models were compared using the receiver operating characteristic (ROC) curves to determine each model's ability to discriminate between those with no/mild periodontitis and those with moderate-to-severe periodontitis. We assessed internal validity with a bootstrapping procedure for a realistic estimate of the performance of the candidate models. We repeated the entire modelling process, including variable selection, in 600 subjects drawn with replacement from the original sample. This approach allows to calculate the loss of prediction (shrinkage), and although presents several weaknesses, it is the recommended internal validation strategy in prediction model studies, as large sample sizes (like the one derived from the NHANES 2011–2012 data set) make this approach reasonable (Steyerberg et al., 2001; Moons et al., 2015). Finally, a prognostic index table was created in order to facilitate the evaluation of any subject in their risk for suffering moderate-to-severe periodontitis.

3 | RESULTS

The sample selected from the NHANES 2011–2012 data set included a total of 3,017 subject records, representing 30.92% of the original national sample. Sample characteristics are presented in Table 1. Briefly, 1,516 males (50.25%) and 1,501 females (49.75%) were included in this study. Mean age was 51.62 ± 14.28 for males and 51.82 ± 14.00 for females. The most frequent ethnicity was non-Hispanic whites (38.18%) followed by African American (24.49%) and Hispanics (21.18%). Mean blood pressure values were 123.92 ± 17.75 mmHg for systolic blood pressure (SBP) and 72.17 ± 12.16 mmHg for diastolic blood pressure (DBP). The mean values for other cardio-metabolic measures were borderline high or high (HbA1c, 5.85 ± 1.17% and total cholesterol, 197.99 ± 41.32 mg/

TABLE 1 Characteristics of the subset of NHANES 2011–2012 sample and prevalence of no/mild, moderate and severe periodontitis

	NHANES 2011–2012	Periodontal status category		
	Sample % (*n*)	No/Mild periodontitis	Moderate periodontitis	Severe periodontitis
Overall	100% (3017)	49.75% (1501)	37.06% (1118)	13.19% (398)
Age	100% (3017)			
30–39	24.76% (747)	71.22% (532)	23.96% (179)	4.82% (36)
40–49	22.41% (676)	54.44%[b] (368)	32.84%[b] (222)	12.72%[b] (86)
50–59	20.88% (630)	44.60%[b] (281)	36.35%[b] (229)	19.05%[b] (120)
60–69	19.22% (580)	34.31%[b] (199)	45.34%[b] (263)	20.34%[b] (118)
70–80	12.73% (384)	31.51%[b] (121)	58.59%[b] (225)	9.90%[b] (38)
Gender	100% (3017)			
Male	50.25% (1516)	41.09% (623)	39.51% (599)	19.39% (294)
Female	49.75% (1501)	58.49%[b] (878)	34.58%[b] (519)	6.93%[b] (104)
Race/Ethnicity	100% (3017)			
Non-Hispanic white[a]	38.18% (1152)	59.64% (687)	32.12% (370)	8.25% (95)
Hispanic	21.18% (639)	42.41% (271)[c]	43.04% (275)[c]	14.55% (93)[c]
African American	24.49% (739)	38.97% (288)[c]	41.41% (306)[c]	19.62% (145)[c]
Asian American	13.52% (408)	51.47% (210)[c]	34.56% (141)	13.97% (57)[c]
Other or multiracial	2.62% (79)	56.96% (45)	32.91% (26)	10.13% (8)
Education	79.54% (2400)			
Secondary school[a]	21.58% (518)	29.92% (155)	49.03% (254)	21.04% (109)
High school graduate	21.50% (516)	38.57% (199)	42.25% (218)	19.19% (99)
College degree	28.08% (674)	52.52% (354)[c]	35.76% (241)[c]	11.72% (79)[c]
College graduate or above	28.83% (692)	66.91% (463)[c]	26.73% (185)[c]	6.36% (44)[c]
Annual household income	77.32% (2333)			
<$20,000	20.02% (467)	34.90% (163)	47.32% (221)	17.77% (83)
$20,000–45,000	28.03% (668)	40.57% (271)	43.56% (291)	15.87% (106)
$45,000–75,000	17.32% (404)	54.21% (219)[c]	32.92% (133)[c]	12.87% (52)
$75,000–100,000	9.73% (227)	63.44% (144)[c]	27.31% (62)[c]	9.25% (21)[c]
>$100,000	19.46% (454)	67.40% (306)[c]	25.33% (115)[c]	7.27 (33)[c]
Smoking habit	100% (3017)			
Non-smoker	56.94% (1718)	57.39% (986)	33.70% (579)	8.91% (153)
Former smoker	24.66% (744)	45.70%[b] (340)	39.38%[b] (293)	14.92%[b] (111)
Smoker	18.40% (555)	31.53%[b] (175)	44.32%[b] (246)	24.14%[b] (134)
BMI (kg/m^2)	100% (3017)			
Underweight/Normal	28.27% (853)	52.52% (448)	33.29% (284)	14.19% (121)
Overweight	35.00% (1056)	49.15% (519)	37.22% (393)	13.64% (144)
Obese	36.73% (1108)	48.19% (534)	39.80% (441)	12% (133)
HbA1c	100% (3017)			
min–5.6%	55.72% (1681)	58.66% (986)	31.23% (525)	10.11% (170)
5.7–6.4%	31.69% (956)	41.00%[b] (392)	43.51%[b] (416)	15.48%[b] (148)
6.5–8%	8.02% (242)	37.19%[b] (90)	45.87%[b] (111)	16.94%[b] (41)
>8%	4.57% (138)	23.91%[b] (33)	47.83%[b] (66)	28.26%[b] (39)
Blood pressure and total cholesterol	Mean ± SD			
Systolic blood pressure (mmHg)	123.92 ± 17.75	120.70 ± 16.30	126.53 ±18.02	128.73 ± 19.86
Diastolic blood pressure (mmHg)	72.17 ± 12.16	72.71 ± 11.18	71.00 ±12.99	73.49 ± 13.02
Total cholesterol (mg/dl)	197.99 ± 41.33	198.31 ± 39.34	196.89 ± 42.35	199.91 ± 45.54

Notes. SD, standard deviation.

[a]Reference category. [b]Statistically significant difference when comparing with the immediate upper category ($p < 0.01$). [c]Statistically significant difference when comparing with the reference category ($p < 0.01$).

TABLE 2 Adjusted associations[b], expressed as odds ratio (95% confidence interval), between demographics and diagnosis of moderate-to-severe periodontitis

Demographic risk factor		Odds ratio (95% confidence interval)
Age	<50 years[b]	
	≥50 years	2.61 (2.12–3.21)[d]
Gender	Female[b]	
	Male	2.21 (1.81–2.69)[d]
Ethnicity	Non-Hispanic white[b]	
	Hispanic	1.58 (1.21–2.06)[d]
	African American	1.91 (1.50–2.44)[d]
	Asian American	2.20 (1.61–3.01)[d]
Educational level	Secondary school[b]	
	High school graduate	0.80 (0.60–1.08)
	College degree	0.54 (0.41–0.72)[d]
	College graduate	0.40 (0.30–0.55)[d]
Household income	<$20,000[b]	
	$20,000–$100,000	0.74 (0.57–0.94)[c]
	>$100,000	0.44 (0.33–0.59)[d]

[a]Considering as confounders the rest of the demographic, socio-economic and lifestyle variables, named: age, gender, smoking habit, ethnicity, educational level and household income. [b]Reference category, Odds ratio = 1. [c]$p < 0.05$. [d]$p < 0.01$.

dl). The number of subjects by BMI category were 853 (28.27%) for underweight/normal body weight, 1,056 (35.00%) for overweight and 1,108 (36.73%) for obese.

The prevalence of moderate and severe periodontitis was 37.06% and 13.19%, respectively. Moderate-to-severe periodontitis prevalence was progressively higher by decade of age and HbA1c levels, and higher in males and smokers (Table 1). Moderate-to-severe periodontitis prevalence was the highest in African Americans (61.0%) and Hispanics (57.6%), followed by Asian Americans (48.5%), and the lowest in Non-Hispanic Whites (40.4%).

Tables 2 and 3 list the socio-demographic variables considered, along with their OR and their CI, obtained from the multiple logistic regression analysis adjusted for the effect of age, gender, educational level, household income, ethnicity and smoking, when using periodontal status and mean CAL as independent variables.

Among the socio-demographic determinants, age was the strongest indicator for having moderate-to-severe periodontitis, as well as for mean CAL 4-6 mm or mean CAL ≥6 mm (OR = 2.61, 95% CI 2.12–3.21, $p < 0.01$; OR = 2.83, 95% CI 2.04–3.90, $p < 0.01$; OR = 8.21, 95% CI 2.99–22.54, $p < 0.01$; respectively), followed by male gender. Hispanic, African American and Asian American ethnicity were statistically associated with periodontal status according to AAP-CDC case definition (OR = 1.58, 95% CI 1.21–2.06, $p < 0.01$; OR = 1.91, 95% CI 1.50–2.44, $p < 0.01$; OR = 2.20, 95% CI 1.61–3.01, $p < 0.01$; respectively) but only African American ethnicity was associated with mean CAL (OR = 1.73, 95% CI 1.22–2.45, $p < 0.01$, for CAL 4–6 mm; OR = 4.27, 95% CI 1.73–10.57, $p < 0.01$, for CAL ≥6 mm). Higher educational level and household income were identified as negatively associated with moderate-to-severe periodontitis.

Regarding the cardio-metabolic risk indicators, and after adjusting for confounders, smoking habit was the strongest indicator, followed by HbA1c. Smoker's OR for having moderate-to-severe periodontitis were 2.91 (95% CI 2.23–3.80) when compared with non-smokers (Table 4). Also, in subjects with HbA1c ≥5.7% (pre-diabetes or diabetes), OR for having moderate-to-severe periodontitis were 1.29 (95% CI 1.07–1.57), when compared with those with normal HbA1c values. In subjects with <50 years, HbA1c values ≥5.7% were associated with an increased OR for suffering moderate-to-severe periodontitis (OR = 1.42, 95% CI 1.05–1.94, $p < 0.05$), and the magnitude of this association increased in the subgroup of smokers (OR = 2.43, 95% CI 1.47–4.01, $p < 0.01$). Obese young adults (<50 years) exhibited an OR of 1.56 (95% CI 1.08–2.26) for presenting moderate-to-severe periodontitis. No significant associations were found for blood pressure (SBP or DBP) or total cholesterol and periodontal status.

For the continuous measure of periodontitis (mean CAL) in fully adjusted models, smoking was again the strongest indicator for mean CAL ≥6 mm (OR = 6.79, 95% CI 2.89–15.98, $p < 0.01$; Table 5). HbA1c ≥5.7% was the only cardio-metabolic parameter significantly associated with mean CAL ≥ 6 mm (OR = 1.43, 95% CI 1.10–1.87, $p<0.01$), as neither BMI, blood pressure nor total cholesterol presented significant associations.

The predictive model with the highest AUC (AUC = 0.801) is always the one comprising all variables: age, gender, ethnicity, HbA1c, BMI, SBP, DBP, total cholesterol, smoking status, educational level and household income. However, this model did not consider the number of variables of the model (11), neither presented the best fit according to the AIC criteria. In order to solve these issues, both AUC and AIC criteria were considered, with a model comprising just five variables (age, gender, ethnicity, HbA1c and smoking habit) presenting a similar AUC, sensitivity and specificity (0.801 vs. 0.773, 73.2% versus 70.0%, 71.2% vs. 67.6%, for the model with 11 variables versus the model with five variables, respectively) but with a lower number of variables involved. The analysis comparing the ROC curves of the maximum and candidate models showed that they were very similar (Figure 1) and using chi-squared tests it was demonstrated that differences between them were not statistically significant ($\chi^2 = 0.228$). The proposed predictive model was tested and validated through a bootstrap validation approach. This analysis found that the model's predictability was reliable, as long as the loss of prediction/shrinkage was 1.00% (result of the difference between the AUC in the entire sample [0.801] and the bootstrap sample [0.791]). The full prediction model is presented in Supporting information Table S2.

Finally, a prognostic index table with the relative risks (RR), for all the possible combinations of the predictive variables, was built in order to facilitate the determination of the risk for suffering moderate-to-advance periodontitis (Appendix S1). The reference pattern (RR = 1) corresponds to a non-Hispanic white non-smoker female

TABLE 3 Adjusted associations[b], expressed as odds ratio (95% confidence interval), between demographics and mean CAL (CAL<4 mm serve as category of reference)

Demographic risk factor		Mean CAL 4–6 mm	Mean CAL ≥6 mm
Age	<50 years[b]		
	≥50 years	2.83 (2.04–3.90)[d]	8.21 (2.99–22.54)[d]
Gender	Female[b]		
	Male	2.58 (1.90–3.50)[d]	5.98 (2.48–14.38)[c]
Ethnicity	Non-Hispanic white[b]		
	Hispanic	1.17 (0.79–1.74)	2.48 (0.86–7.19)
	African American	1.73 (1.22–2.45)[d]	4.27 (1.73–10.57)[d]
	Asian American	1.65 (0.99–2.73)	1.55 (0.36–6.72)
Educational level	Secondary school[b]		
	High School graduate	0.70 (0.49–0.99)[c]	1.16 (0.53–2.56)
	College degree	0.40 (0.27–0.58)[d]	0.27 (0.09–0.79)[c]
	College graduate	0.19 (0.11–0.32)[c]	0.52 (0.16–1.64)
Household income	<$20,000[b]		
	$20,000–$100,000	0.69 (0.50–0.94)[c]	0.91 (0.44–1.90)
	>$100,000	0.44 (0.28–0.69)[d]	0.34 (0.10–1.16)

[a]Considering as confounders the rest of the demographic, socio-economic and lifestyle variables, named: age, gender, smoking habit, ethnicity, educational level and household income. [b]Reference category, Odds ratio = 1. [c]$p < 0.05$. [d]$p < 0.01$.

between 30–40 years with HbA1c ≤5.7%. The highest RR (RR = 9.91) corresponds to a Hispanic, male, smoker, between 70–80 years old and presenting HbA1c ≥6.5%.

4 | DISCUSSION

The results from this cross-sectional study provide evidence of the significant relationship between cardio-metabolic risk measures and the prevalence of moderate-to-severe periodontitis in the U S adult population. It further provides the basis for using algorithms integrating demographic, lifestyle and cardio-metabolic measures to screen for periodontitis in patients examined in primary medical care settings.

The prevalence of moderate-to-severe periodontitis in this subset of the 2011–2012 NHANES data set (participants aged >30 years with >14 teeth having received a periodontal examination, as well as having age, gender, ethnicity, smoking habit, BMI, blood pressure,

TABLE 4 Adjusted associations[b], expressed as odds ratios (95% confidence interval), between individual cardio-metabolic risk factors and diagnosis of moderate-to-severe periodontitis

Cardio-metabolic risk factor		All sample (n = 3017)	<50 years (n = 1423)	Smokers (n = 555)
Smoking habit	Non-smoker[b]			
	Former smoker	1.22 (0.97–1.54)	1.38 (0.92–2.08)	–
	Smoker	2.91 (2.23–3.80)[d]	2.57 (1.78–3.69)[d]	–
HbA1c	<5.7%[b]			
	≥5.7%	1.29 (1.07–1.57)[d]	1.42 (1.05–1.94)[c]	2.43 (1.47–4.01)[d]
BMI	<25 kg/m^2			
	Overweight	1.10 (0.86–1.40)	1.14 (0.78–1.66)	1.26 (0.71–2.24)
	Obesity	1.26 (0.99–1.60)	1.56 (1.08–2.26)[d]	1.37 (0.77–2.44)
SBP	<140 mm Hg[b]			
	≥140 mm Hg	1.16 (0.89–1.50)	1.48 (0.88–2.50)	1.26 (0.61–2.59)
DBP	<90 mm Hg[b]			
	≥90 mm Hg	1.15 (0.79–1.67)	1.36 (0.82–2.26)	1.08 (0.47–2.49)
Total cholesterol	<200 mg/dl[b]			
	≥200 mg/dl	1.02 (0.85–1.23)	0.82 (0.62–1.10)	1.12 (0.70–1.79)

Notes. BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin; SBP, systolic blood pressure.
[a]Considering as confounders the rest of the demographic, socio-economic and lifestyle variables, named: age, gender, smoking habit, ethnicity, educational level and household income. [b]Reference category, Odds ratio = 1. [c]$p < 0.05$. [d]$p < 0.01$.

TABLE 5 Adjusted associations[b] between individual cardio-metabolic risk factors and mean CAL≥6 mm

Cardio-metabolic risk factor		All sample (n = 3017)	<50 years (n = 1423)	Smokers (n = 555)
Smoking habit	Non-smoker[b]			
	Former smoker	1.83 (0.76–4.45)	0.97 (0.25–3.76)	–
	Smoker	6.79 (2.89–15.98)[d]	2.73 (1.18–6.35)[c]	–
HbA1c	<5.7%[c]			
	≥5.7%	1.43 (1.10–1.87)[d]	2.08 (0.87–4.93)	1.46 (0.84–2.15)
BMI	<25 kg/m^2			
	Overweight	0.84 (0.61–1.15)	0.71 (0.37–1.35)	0.68 (0.39–1.17)
	Obesity	0.81 (0.59–1.13)	0.71 (0.37–1.38)	0.72 (0.40–1.28)
SBP	<140 mm Hg[b]			
	≥140 mm Hg	0.90 (0.58–1.40)	0.73 (0.31–1.73)	0.92 (0.42–2.05)
DBP	<90 mm Hg[b]			
	≥90 mm Hg	1.43 (0.73–2.42)	1.54 (0.71–3.30)	1.43 (0.66–3.11)
Total cholesterol	<200 mg/dl[b]			
	≥200 mg/dl	1.16 (0.90–1.51)	1.36 (0.68–1.90)	1.60 (0.99–2.58)

Notes. BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin; SBP, systolic blood pressure.
[a]Considering as confounders the rest of the demographic, socio-economic and lifestyle variables, named: age, gender, smoking habit, ethnicity, educational level and household income. [b]Reference category, OR = 1. [c]$p < 0.05$. [d]$p < 0.01$.

total cholesterol and HbA1c registered) was ≈50%, with 13.19% having severe periodontitis and 37.06% moderate periodontitis. These findings are similar to those reported by Eke et al. (Eke et al., 2015) when combining NHANES 2009 to 2012 data. They reported that ≈46% of US dentate adults had periodontitis, with 8.9% having severe periodontitis and 37.1% having less severe forms (Eke et al., 2015).

These results also confirm the reported disparities in the burden of periodontitis according to the different socio-demographic segments of the population. Among ethnic groups, Hispanic and African American populations showed the highest prevalence of periodontitis, while Asian Americans and Non-Hispanic Whites had the lowest. The prevalence of moderate-to-severe periodontitis also increased with the decrease in educational levels and annual household income. These socio-economic and demographic patterns together with the identification of current smoking as the most important risk indicator were consistent with previous findings from NHANES, and reinforce the need to adjust for confounders when evaluating the association between cardio-metabolic risk factors and periodontitis (Albandar, Brunelle, & Kingman, 1999; Tomar & Asma, 2000; Eke, Dye, Wei, Thornton-Evans, & Genco, 2012).

Mean values for cardio-metabolic risk measures were higher (although non-statistically significant) in the subset of individuals included in this study, when compared with the NHANES data set, which may be in part explained by the inclusion of only >30 years of age in the study sample (Supporting information Table S1). In this subset, there was a significant association between HbA1c levels and moderate-to-severe periodontitis, as well as between HbA1c levels and attachment levels in adult individuals. It has been long known that there is a two-way relationship between periodontitis and diabetes, as inflammation is a central feature of both diseases. Recent evidence indicates that high levels of IL-1β, TNF-α and IL-6 are present in gingival tissues in poorly controlled diabetes subjects and that periodontitis worsens glycaemic control and leads to circulating elevated levels of these and other systemic inflammatory mediators such as C-reactive protein (Polak & Shapira, 2018). The results of this study highlight the importance of collecting information on blood glucose levels during periodontal diagnosis, which may result in identification of undiagnosed pre-diabetes or diabetes and better individualized treatment regimens for patients with diabetes and periodontitis (Lalla, Kunzel, Burkett, Cheng, & Lamster, 2011; Dye & Genco, 2012; Lalla, Cheng, Kunzel, Burkett, & Lamster, 2013). Moreover, in the light of mounting evidence indicating that periodontal therapy can improve HbA1c levels by up to 0.40% (Engebretson & Kocher, 2013), these findings are of particular importance. It is estimated that 1% reduction in HbA1c levels in diabetic patients results in 35% reduction in the risk of cardiovascular complications (Stratton et al., 2000) and that a 0.2% HbA1c reduction is associated with a 10% reduction in mortality in the general population (Khaw et al., 2004).

Although BMI within the multivariate analyses was not an important predictor for periodontitis, the ORs for obese young adults to present moderate-to-severe periodontitis were significantly higher after adjustment for all confounders. This fact is in agreement with the evidence derived from numerous studies showing an association between obesity and periodontitis (Suvan et al., 2011; Chaffee & Weston, 2010). In a long-term longitudinal study (30 years), on the progression of periodontitis and body adiposity in men, Gorman et al. found that both subcutaneous and visceral adiposity increases were associated with periodontitis progression

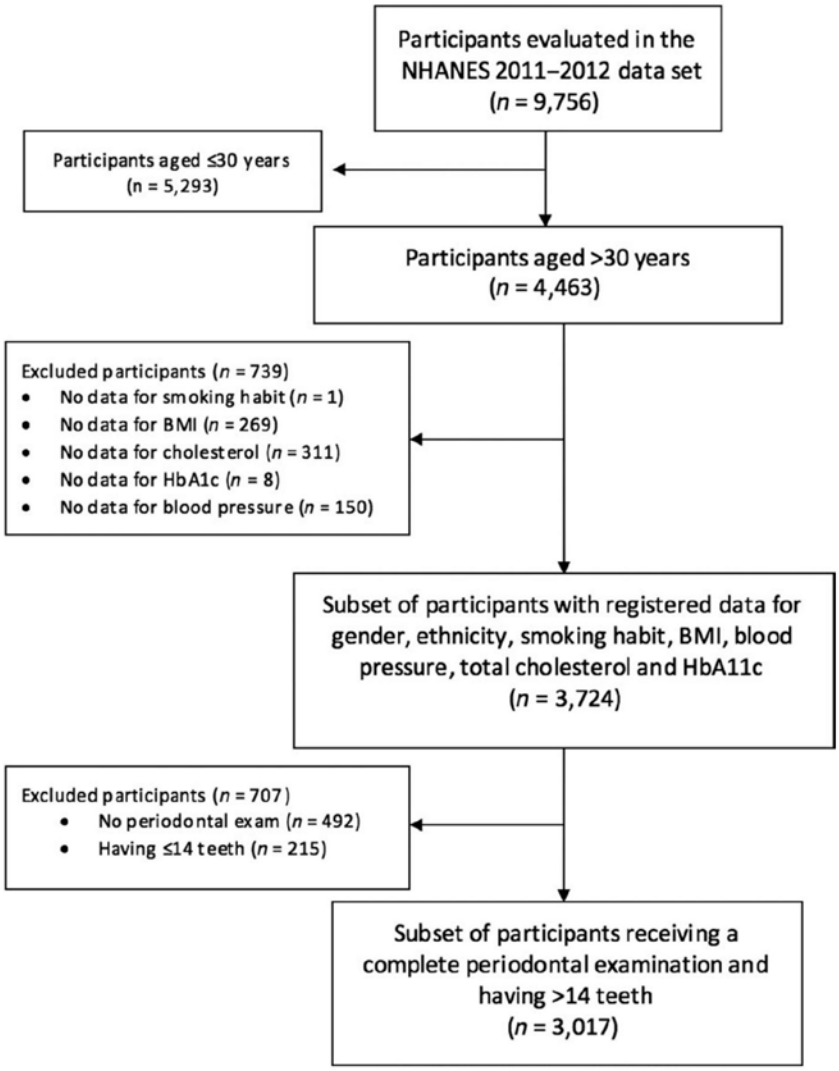

FIGURE 1 Flow chart indicating the subset of participants included for the analysis from the NHANES 2011–2012 data set

(Gorman, Kaye, Nunn, & Garcia, 2012). In the present study, a significant association was found only for subjects <50 years, supporting that a stronger association between periodontitis and obesity may occur on younger individuals, mainly in women and non-smokers (Chaffee & Weston, 2010). Other studies have reported that waist circumference and waist-to-hip ratio correlated stronger than BMI with periodontitis (Al-Zahrani, Bissada, & Borawskit, 2003; Wood, Johnson, & Streckfus, 2003; Kim, Jin, & Bae, 2011). This is partly explained by BMI not distinguishing between visceral and subcutaneous adiposity, and the former is the main source of pro-inflammatory adipokines that prime for increased systemic inflammatory tone.

Using a representative national US sample of adults >30 years old, we have developed a predictive risk model for moderate-to-severe periodontitis. However, due to the cross-sectional nature of this study, periodontitis disease activity or progression as well as the history of exposure to cardio-metabolic risk factors could not be assessed and, hence, the possible inferences about the direction of the reported relationships cannot be made. It is also important to consider that the predictive model is applicable mainly to those subjects not visiting a dentist regularly. Unfortunately, this a frequent finding in the study population, as 42.09% of the participants did not

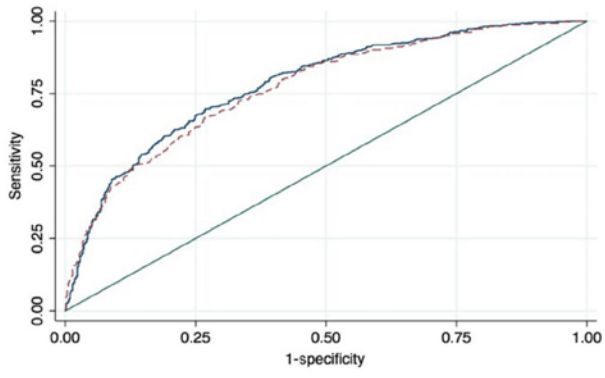

FIGURE 2 Graphic representation of the receiver operating characteristic (ROC) curves of the maximum (straight blue line) and proposed (dash maroon line) predictive models

attend to the dentist in the last year. Moreover, just attending the dentist does not necessarily imply that proper periodontal diagnosis has been made, as non-recognition of periodontitis is a common cause of professional litigation (Zinman, 2001). Another limitation is that the predictive model is applicable to the US population and cannot be extrapolated to other nations with different socio-economic demographics and healthcare systems. For these reasons, the proposed predictive model, or similar ones based on socio-demographic and cardio-metabolic risk factors, needs future validation in others, if possible, prospective cohorts such as the Study of Health in Pomerania (SHIP-Trend), or other data sets from NHANES.

In conclusion, our findings support the concept of personalized integrative approaches by physicians and periodontists in the management of cardio-metabolic disorders and periodontitis. The predictive model developed and validated to screen for existing undiagnosed and untreated moderate-to-severe periodontitis, using a combination of cardio-metabolic (HbA1c), demographic (age, gender and ethnicity) and lifestyle variables (smoking habit), may be used by physicians in primary medical care settings. However, further validation of this model across different populations is needed for development of guidelines and applicability in non-US populations. This study further reinforces the need for guidelines to screen for periodontitis, pre-diabetes and diabetes in dental and medical care settings. Measures of modifiable risk factors for periodontitis and diabetes, such as glycaemic control and adiposity, seem to be universally applicable screening parameters.

CONFLICT OF INTEREST

Authors declare no conflicts of interest in relation to this study.

AUTHOR CONTRIBUTIONS

EM designed the study, performed data analysis and wrote the manuscript. DH, MS and SD critically reviewed the manuscript. TVD designed the study and critically reviewed the manuscript. CS designed the study and wrote the manuscript. All authors have seen and approved the final version of the manuscript. EM and CS are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

ORCID

Eduardo Montero https://orcid.org/0000-0003-2525-8529
Corneliu Sima https://orcid.org/0000-0003-3526-4850

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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STUDY #5

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Screening of undiagnosed hyperglycemia in the dental setting: the DiabetRisk study.

Aim: To evaluate the efficacy of different screening protocols for undiagnosed hyperglycaemia in a Research Network of Dental Clinics coordinated by the Spanish Society of Periodontology (SEPA).

Material and Methods: A total of 1143 subjects were included in the study. Participants filled a questionnaire considering diabetes risk factors (FINDRISC) and received a periodontal screening examination. Subjects with a slightly elevated score according to the FINDRISC (≥7), received a point-of-care HbA1c and were eventually referred to their physician for confirmatory diagnosis. Receiver Operating Characteristic (ROC) curves were used to assess the performance of various predictive models with confirmed hyperglycaemia as outcome.

Results: From this population, 97 (8.5%) were finally diagnosed of diabetes (n=28; 2.5%) or prediabetes (n=69; 6.0%). When only including the results from the FINDRISC questionnaire, the model reported an area under the curve (AUC) of 0.866 (95% confidence interval - CI [0.833; 0.900]). This model significantly improved when the point-of-care HbA1c was added (AUC= 0.961; 95% CI [0.941; 0.980]; p<0.001).

Conclusions: The tested protocol, combining the FINDRISC questionnaire and a point-of-care HbA1c, showed to be feasible when carried out in a dental clinic setting and was efficient to identify subjects with undiagnosed diabetes or prediabetes.

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Screening of Undiagnosed Hyperglycaemia in the Dental Setting: the *DiabetRisk* Study

Authors: Eduardo Montero[1,2], Paula Matesanz[1,4], Antonio Nobili[1], José Luis Herrera-Pombo[2,3], Mariano Sanz[1,4], Adrián Guerrero[4], Antonio Bujaldón[4], David Herrera[1,2,4], on behalf of the SEPA Research Network of Dental Clinics*

1. ETEP (Etiology and Therapy of Periodontal and Peri-implant Diseases) Research Group, University Complutense of Madrid, Spain
2. Working Group "Diabetes and Periodontal Diseases" of the Spanish Society of Diabetes (SED) and the Spanish Society of Periodontology (SEPA)
3. Endocrinology and Nutrition Department. University Hospital Fundación Jiménez Díaz, Madrid, Spain
4. *Fundación SEPA de Periodoncia e Implantes Dentales* and Spanish Society of Periodontology (SEPA), Madrid, Spain.

Short title: Diabetes Screening in the Dental Setting

Corresponding author:
Eduardo Montero
Faculty of Odontology (University Complutense of Madrid)
Plaza de Ramón y Cajal s/n (Ciudad Universitaria)
28040 Madrid
Spain
Email: eduardomonterosolis@ucm.es

***SEPA Research Network of Dental Clinics**
Clínica Laura Campos, Zaragoza, Spain. Dra. Laura Campos.
Clínica Mateos, Sevilla, Spain. Dr. Leopoldo Mateos.
Clínica Blanco-Ramos, Santiago de Compostela, Spain. Dra. Pilar Batalla.
Master de Periodoncia, Universidad de Santiago de Compostela, Santiago de Compostela, Spain. Dr. Juan Blanco.

Clínica Enrile, Huelva, Spain. Dr. Francisco Enrile.

Clínica Vijande, Bilbao, Spain. Dr. Francisco Vijande.

Clínica Ortiz-Vigón, Bilbao, Spain. Dr. Alberto Ortiz-Vigón.

Clínica Álvarez-Novoa, Santander, Spain. Dra. Carmen Álvarez-Novoa.

Clínica Periodontal Dr. Bujaldón, Almería, Spain. Dr. Juan Contreras Cano.

Clínica Alcaraz, Alicante, Spain. Dr. Jaime Alcaraz Freijo.

Clínica Antonio Liñares, A Coruña, Spain. Dr. Antonio Liñares.

Albia Clínica Dental, Bilbao, Spain. Dr. Ion Zabalegui.

Periodoncia Ciudad Real, Ciudad Real, Spain. Dra. Cristina Serrano.

Master de Periodoncia, Universidad Católica de Valencia, Valencia, Spain. Dr. Pedro Buitrago.

Master de Periodoncia, Universidad Complutense de Madrid, Madrid, Spain. Dr. Mariano Sanz.

Periocentrum Segovia, Segovia, Spain. Dr. Alfonso Oteo.

Clínica Blanco-Moreno, Oviedo, Spain. Dr. Fernando Blanco-Moreno.

Santvidental, Sant Vicenç de Montalt, Barcelona, Spain. Dra. Angels Pujol.

Clínica Perios, Valencia, Spain. Dra. Amparo Lorca.

Clínica Carrasquer de odontología integrada, Valencia, Spain. Dra. Assumpta Carrasquer.

Instituto Maxilofacial, Centro Médico Teknon, Barcelona, Spain. Dra. Vanessa Ruiz.

Clínica Dental Serrano, Madrid, Spain. Dr. Jorge Serrano.

Centro Avanzado de Odontología Belmonte, Albacete, Spain. Dr. Nicolás Belmonte.

Clínica Dental Pico Blanco, Carballo, A Coruña, Spain. Dr. Alex Pico.

Clínica Dental Sanchez Cortés, Pozuelo de Alarcón, Madrid. Dra. María Rioboo.

Clínica Dorado y Mateos, Murcia, Spain. Dra. Bibiana Mateos.

Perioten, Santa Cruz de Tenerife, Spain. Dr. Antonio Gómez.

Clínica Periodontia, Gijón, Spain. Dra. Susana Cuesta.

Centre Dental Amat, Barcelona, Spain. Dra. Gloria Calsina.

Clínica Dra. Sanz, Vigo, Spain. Dra. Adriana Sanz.

Unidad de Periodoncia, Complejo Hospitalario Ruber Juan Bravo, Madrid, Spain. Dr. Miguel Carasol

Clínica Flores Carral, Toledo, Spain. Dra. Cristina Carral.

Clínica Dental Segundo Rodríguez Grandío, Vigo, Spain. Dr. Segundo Rodríguez Grandío.

Master de Periodoncia, Universidad de Sevilla, Sevilla, Spain. Dr. Pedro Bullón.

Universidad de Granada, Granada, Spain. Dr. Gerardo Gómez Moreno.

Clínica Alonso, Salamanca, Spain. Dra. Ana Molina.

Clínica Izquierdo, Gandía, Valencia, Spain. Dra. Regina Izquierdo.

Clínica Periodontium, Santander, Spain. Dr. Alberto Remolina.

Centro Dental Cordero, Huelva, Spain. Dr. Alberto Gómez Menchero.

Policlínica Dr. Merino, Ponferrada, León, Spain. Dr. Enrique Merino.

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ABSTRACT

Aim: To evaluate the efficacy of different screening protocols for undiagnosed hyperglycaemia in a Research Network of Dental Clinics coordinated by the Spanish Society of Periodontology (SEPA).

Material and Methods: A total of 1143 subjects were included in the study. Participants filled a questionnaire considering diabetes risk factors (Findrisc) and received a periodontal screening examination. Subjects with a slightly elevated score according to the Findrisc (≥7), received a point-of-care HbA1c and were eventually referred to their physician for confirmatory diagnosis. Receiver Operating Characteristic (ROC) curves were used to assess the performance of various predictive models with confirmed hyperglycaemia as outcome.

Results: From this population, 97 (8.5%) were finally diagnosed of diabetes (n=28; 2.5%) or prediabetes (n=69; 6.0%). When only including the results from the Findrisc questionnaire, the model reported an area under the curve (AUC) of 0.866 (95% confidence interval - CI [0.833; 0.900]). This model significantly improved when the point-of-care HbA1c was added (AUC= 0.961; 95% CI [0.941; 0.980]; p<0.001).

Conclusions: The tested protocol, combining the Findrisc questionnaire and a point-of-care HbA1c, showed to be feasible when carried out in a dental clinic setting and was efficient to identify subjects with undiagnosed diabetes or prediabetes.

Keywords: diabetes, periodontitis, prediabetes, screening, prevention, undiagnosed diabetes

CLINICAL RELEVANCE

Scientific rationale for study: Screening for hyperglycaemia has shown efficacy to prompt for early diagnosis and treatment and thus, reducing the cardiovascular risk of affected subjects. Dental offices have been proposed as a suitable setting to screen for subjects at risk.

Principal findings: The combination of the Findrisc questionnaire and a point-of-care HbA1c was efficient in identifying subjects with undiagnosed diabetes or prediabetes. The addition of periodontal parameters as predictors did not significantly improved the screening capacity of the tested models.

Practical implications: The oral care team should be involved in the screening of undiagnosed hyperglycaemia to facilitate early diagnosis and management of diabetes and prediabetes.

Introduction

Diabetes mellitus (DM) is a chronic non-communicable disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. The World Health Organization (WHO) estimates that 422 million adults worldwide suffer from this disease, and more than 3 million die annually as a result of their hyperglycaemia (World Health Organization, 2016).

In Spain, DM has a high prevalence (12.5%) (Valdes et al., 2014), and high mortality rate (around 20 deaths per 100.000 people/year in males and 25 deaths per 100,000 persons/year in women) (Ruiz-Ramos, Escolar-Pujolar, Mayoral-Sanchez, Corral-San Laureano, & Fernandez-Fernandez, 2006). Although the epidemiological data on type 2 DM alone are scarce, the estimates from the di@bet.es study report a prevalence of 13.8% of the adult population (5,301,314 people), including 6% of the population being undiagnosed (2,304,919 people) (Soriguer et al., 2012). It is estimated that only in Spain, DM costs 5.1 billion euro in direct costs, and 1.5 billion euro in indirect costs associated with the management of diabetes complications (Lopez-Bastida, Boronat, Moreno, & Schurer, 2013), what clearly underpins the need to have effective strategies for the early detection, prevention and treatment of this disease.

Periodontitis is a chronic non-communicable inflammatory disease characterized by the destruction of the tooth-supporting tissues. It is considered one of the most prevalent human diseases with a world prevalence of around 800 million (Global Burden of Disease Oral Disorders Collaborators et al., 2020). In Spain, around 8% of the employed adult population present severe forms of periodontitis (Carasol et al., 2016). Furthermore, periodontitis has demonstrated to contribute to the systemic inflammatory burden, being associated with the majority of chronic non-communicable diseases (Genco & Sanz, 2020).

Different reviews and consensus documents published in recent years have clearly indicated the bidirectional association between diabetes and periodontitis (Chapple, Genco, & Working group 2 of joint, 2013; Preshaw et al., 2012), demonstrating that DM (both types 1 and 2) is a risk factor for periodontitis (Nelson et al., 1990; Taylor et al.,

1998) and that periodontitis negatively affects glycaemic control in patients with diabetes, significantly contributing to the onset of complications (Demmer et al., 2010; Ide, Hoshuyama, Wilson, Takahashi, & Higashi, 2011; Shultis et al., 2007). These findings highlight the important implications of the association between DM and periodontitis for health professionals, diabetic patients, periodontitis patients and the general population.

Prediabetes, characterized by mild hyperglycaemia and a slight increase in insulin resistance, often precedes type 2 DM, and it is considered an independent risk factor for cardiovascular disease (Tabak, Herder, Rathmann, Brunner, & Kivimaki, 2012). However, this conversion from prediabetes to diabetes is not inevitable, and its early identification and the implementation of preventive measures may reduce its onset and the risk of further complications. Early diagnosis of DM, therefore, is of paramount importance in the mitigation of diabetes complications and its associated co-morbidities (American Diabetes Association, 2014).

Multiple predictive models have been tested in the search for the most appropriate method of detecting undiagnosed diabetes. These methods have used combinations of patient-reported questionnaires with objective measures, such as age, body mass index (BMI), etc. (Kahn, Cheng, Thompson, Imperatore, & Gregg, 2009; Lindstrom & Tuomilehto, 2003; Wilson et al., 2007). In fact, the joint workshop on periodontal diseases and diabetes, organized by the International Diabetes Federation (IDF) and the European Federation of Periodontology (EFP) in 2017, concluded that screening protocols for type 2 DM performed in dental clinics are effective (Sanz et al., 2018). It was, therefore, the objective of the present study to evaluate the efficacy of protocols for risk assessment in the detection of undiagnosed diabetes or prediabetes in a network of dental clinics, carried out by oral health professionals.

Material and Methods

Study Design

The *DiabetRisk* study was designed as an observational cross-sectional clinical investigation, aimed to evaluate the ability of a protocol to differentiate between healthy and undiagnosed diabetes or prediabetes subjects. The experimental design of the study, outlined in Figure 1, was performed in accordance with the Declaration of Helsinki on human studies and approved by an institutional ethics committee (Internal Code 16/402-E, Hospital Clínico San Carlos, Madrid). This study conforms with the Standards for the Reporting of Diagnostic Accuracy Studies (STARD) guidelines.

Study Centers and Patient Sample

The study was carried out in a Research Network of Dental Clinics coordinated by the Spanish Society of Periodontology (SEPA). This network gathers university and private dental clinics, under the supervision of dentist with periodontal training at specialist level, willing to participate in practice-based research. The personnel from the clinics, who accepted to participate in the *DiabetRisk* study were trained both at in-person (May 25, 2017) and/or on-line meetings (July 12, 2017) and were in constant contact with the study coordinators during the course of the study. In these participating clinics, study subjects were recruited among consecutive patients attending the clinics and fulfilling the following inclusion criteria: i) age ≥40 years; and ii) never have been diagnosed with diabetes or prediabetes. Pregnant women and patients immunosuppressed or under immunosuppressive treatment were excluded.

Examination Protocol

Once the selected patients agreed to participate in the study and signed the informed consent, a medical history focusing on tobacco smoking, history of cardiovascular diseases (acute myocardial infarction, angina pectoris, peripheral arterial disease, stroke, etc.), renal failure and drug use (mainly focused on diabetogenic agents such as diuretics or corticosteroids) was taken.

Participants were then asked to fill the self-reported Findrisc questionnaire under the supervision of the staff of the clinic (Lindstrom & Tuomilehto, 2003). This questionnaire consists of a series of questions including age, consumption of fruits and vegetables, physical exercise, family history of DM, etc.) The BMI was determined and the

abdominal perimeter (waist circumference, WC) measured. With this information, diabetic risk was calculated using an adapted version prepared by the Spanish Society of Diabetes (SED, *Sociedad Española de Diabetes*; see https://www.fundaciondiabetes.org/prevencion/findrisk).

All participants received a periodontal screening examination (EPB, *Examen Periodontal Básico*) (Serrano & Herrera, 2011), based on the Basic Periodontal Exam developed by the British Society of Periodontology (Tugnait, Clerehugh, & Hirschmann, 2004) and those subjects with codes 3, 4 or * were further assessed by means of a complete periodontal examination, including measurements of probing depth (PD), gingival recession (REC), bleeding on probing (BOP) and plaque (PII, dichotomously), in six sites per tooth, excluding third molars.

Those subjects with a slightly elevated diabetes risk, according to the Findrisc questionnaire (scores ≥7), received a point-of-care glycated haemoglobin (HbA1c) examination using fingerstick blood, analysed with a portable device (A1CNow+®, Bayer Healthcare, Leverkusen, Germany). This test has been certified by both the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (National Glycohemoglobin Standardization Program, 2013).

Participants with HbA1c values ≥5.7% were prompted to visit their physician for confirmation of diagnosis. To support this advice, a letter reporting the results of the screening was given to the patients, who were asked to report back the result of the diagnosis to the dental clinic. During the course of the study, the Spanish Society of Diabetes (SED) and the Spanish Society of Primary Care Physicians (SEMERGEN) informed their members on the purpose of the *DiabetRisk* study and the importance to participate.

Case Definition

The following case definition was used (American Diabetes Association, 2014):

• Diabetes, if Fasting Plasma Glucose (FPG) was ≥ 126 mg/dl or HbA1c was ≥ 6.5%.

• Pre-diabetes, if FPG was between 100-125 mg/dl or HbA1c was between 5.7-6.4%.

Data Collection

The Findrisc questionnaire was filled in by the patient, the periodontal examination and the determination of HbA1C levels were carried out by the responsible oral health provider at each clinic. The results from the diagnostic report by the physician were sent in writing to the dental clinic. All collected data were sent to the study coordinating centre at the University Complutense of Madrid (Spain), where data were organized and analysed.

Sample Size Calculation and Statistical Analysis

It was estimated that the minimum sample size required, based on the prevalence of undiagnosed diabetes in Spain (approximately 6%) (Soriguer et al., 2012), and considering a "fair" sensitivity for a screening test (70%), a statistical power of 0.8 and a significant p value <0.05, was 980 subjects (Bujang & Adnan, 2016).

The quantitative variables were presented as mean ± standard deviation (SD), while the categorical variables were expressed as percentages. The analysis of the variance (ANOVA) was used to compare the quantitative variables, while the Chi-square test was used to compare categorical variables among the three groups (metabolic health, prediabetes, and diabetes).

Receiver Operating Characteristic (ROC) curves were used to assess the performance of various predictive models, using multifactorial models of logistic regression, with confirmed hyperglycaemia (prediabetes or diabetes) as outcome. The diagnostic models evaluated were i) Findrisc alone; ii) EPB alone; iii) Findrisc and EPB; iv) Findrisc and point-of-care HbA1c; v) EPB and point-of-care HbA1c; and vi) Findrisc and point-of-care HbA1c and EPB. Wald 95% confidence intervals (CI) limits for the area under the ROC curve (AUC) were reported. Post-hoc analyses for the predictive capacity of different diabetes risk factors (e.g. BMI, WC), clinical periodontal parameters, or combinations of them were performed. The areas under the curves were compared using the DeLong, DeLong and Clark-Person test (DeLong, DeLong, & Clarke-Pearson, 1988). Optimal cut-offs for

the variables in the preferred models were identified and performance parameters [i.e. sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)] were calculated. For all analyses, a two-sided p value of less than 0.05 was considered as significant. The analyses were carried out using STATA version 13.1 (StataCorp College Station, TX, USA).

Results

Patient sample

The study flow-chart is outlined in Figure 1. From June 2017 to April 2019, the *DiabetRisk* study included 1,143 individuals older than 40 years (out of 1,326 screened) who had never received a diagnosis of diabetes or prediabetes, in 41 clinics from 26 different cities belonging to the SEPA Research Network of Dental Clinics (36 private clinics and 5 university clinics).

From this studied sample, 97 subjects (8.5%) received a confirmatory diagnosis of diabetes (n=28; 2.5%) or prediabetes (n=69; 6.0%), while 23 subjects (2.0%) were identified as potentially diabetic/prediabetic, either because they did not consult with the physician, a blood sample analyses was not done or the patient did not return to the dental clinic.

Findrisc and its components

Information on age, gender, smoking status, Findrisc global score and its individual items, as well as the results from the point-of-care HbA1c, according to their confirmed glycaemic status, is presented in Table 1. Findrisc score was significantly associated with worsened glycaemic control ($p<0.001$). Similarly, subjects with diagnosis of diabetes or prediabetes had a significantly higher BMI when compared with those being metabolically healthy [30.3 kg/m^2 (SD=4.9), 28.37 kg/m^2 (SD=4.7) and 24.9 kg/m^2 (SD=4.3), respectively ($p=0.013$)]. Other individual components of the Findrisc questionnaire, also demonstrating statistically significant differences between groups, were WC (only in women; $p=0.004$), and current or past use of medication to control

blood pressure (p=0.012). Similarly, the results from point-of-care HbA1c showed statistically significant differences between subjects with diabetes or prediabetes when compared with those in metabolic health [6.9 (SD=0.7), 6.0 (SD=0.3) and 5.4% (SD=0.4), respectively, (p=0.040)].

Periodontal evaluation

The proportion of included subjects with EPB code 4 (presence of, at least, one site with PD≥ 6 mm) was significantly higher among subjects in the prediabetes and diabetes groups, compared with the subjects with metabolic health (p<0.001; Table 2). Subjects with a confirmed diagnosis of DM had significantly higher mean PDs and percentages of sites with PPD> 6 mm than subjects with metabolic health or prediabetes.

Predictive models

Six predictive models were evaluated to assess their capacity to detect patients with undiagnosed hyperglycaemia (prediabetes or diabetes). The obtained ROC curves for these models are depicted in Figure 2. Model I considered the results of Findrisc score alone and Model II the highest EPB code. The respective AUC values were 0.866 (n= 1143; 95% CI [0.833; 0.900]) and 0.617 (n=1143; 95% CI [0.588; 0.645]), demonstrating a statistically significant better prediction for Model 1 (p<0.001). Model III combined the Findrisc score with the highest code of EPB, attaining an AUC value of 0.876 (n=1143; 95% CI [0.845; 0.906]), which performed significantly better than Model I (p=0.042) and Model II (p<0.001).

Models IV and V added the point-of-care HbA1c to Findrisc and EPB, respectively. Their respective AUC values were 0.961 (n=399; 95% CI [0.941; 0.980]) and 0.958 (n=399; 95% CI [0.937; 0.978]), demonstrating higher diagnostic capability when compared with Models I, II and III (p<0.001, for all comparisons). Model VI combined Findrisc, EPB and point-of-care HbA1c, attaining an AUC of 0.962 (n=399; 95% CI [0.943; 0.981]), also significantly improving Models I, II and III (p<0.001, for all comparisons). There were no statistically significant differences when comparing models using point-of-care HbA1c (Models IV, V and VI).

Table 3 depicts the optimal cut-offs for the variables included in these models and calculated performance parameters. For the Findrisc score, the cut off was 11 with 31.6% of the participants (25.6% in the metabolic health group and 81.4% in prediabetes or diabetes groups) above the cut-off. For the EPB codes, the cut off was 4, and 37.3% of the participants (34.8% in the metabolic health group and 52.6% in the prediabetes or diabetes groups) were above the threshold. For point-of-care HbA1c the cut off was 5.8%. A Findrisc score of ≥11 correctly identified 81% of the cases with previously undiagnosed hyperglycaemia, with a relatively low probability to detect "diseased" subjects when reporting a positive result (PPV=22%). When point-of-care HbA1c (≥5.8%) was added, the sensitivity increased to 86% and the PPV to 95%. The inclusion of periodontal information (EPB codes) did not significantly improve the performance of the tested screening protocols.

Discussion

The findings from the present investigation (*DiabetRisk* study) demonstrate that dental settings are a suitable environment for the detection of previously unknown diabetes or prediabetes, as 8.5% of the patients attending the aforementioned centres, in demand of dental care, received a confirmatory diagnosis for their hyperglycaemic state. These findings highlight the important role that oral health professionals could play in the early detection of DM, as previously stated in the guidelines derived from the IDF-EFP joint workshop (Sanz et al., 2018). Furthermore, it has been proved that screening for patients with abnormal glucose tolerance is feasible in different settings related to dental practice, from academic centres to private practices.

Similar data of prevalence (≈3%) for undiagnosed diabetes, in patients seeking dental treatment in the oral health setting, have been reported in other studies in Denmark (Holm et al., 2016) and Japan (Harase et al., 2015). However, these prevalence figures are clearly lower than the ones reported by (Lalla, Cheng, Kunzel, Burkett, & Lamster, 2013) for undiagnosed diabetes (8.6%) or undiagnosed prediabetes (46.5%) using HbA1c determinations by HPLC (High Performance Liquid Chromatography), or to the ones using FPG for the diagnosis in a similar study (4.2% and 31.8%, respectively) (Lalla,

Kunzel, Burkett, Cheng, & Lamster, 2011). These differences may be easily attributed to the sample characteristics, since in the studies performed by Lalla et al. (Lalla et al., 2013; Lalla et al., 2011), only patients with, at least, one diabetes risk factor (family history of diabetes, hypertension, high cholesterol or overweight/obesity) were included, while in the *DiabetRisk* study, all subjects over 40 years old were recruited, independently of their risk for suffering undiagnosed hyperglycaemia, what confers higher external validity to this investigation. In addition, the relevant differences in diabetes incidence between Spain and U.S.A. should also be considered (Abraham, Pencina, Pencina, & Fox, 2015; Rojo-Martinez et al., 2020).

The Findrisc questionnaire is one of the most widely used tools to evaluate the risk to develop type 2 diabetes mellitus (Lindstrom & Tuomilehto, 2003; Salinero-Fort et al., 2016; Stiglic, Fijacko, Stozer, Sheikh, & Pajnkihar, 2016), and its use has shown a good screening capacity (AUC=0.87) to discriminate between health and prediabetes/diabetes states, with an optimal cut-off established at a score ≥11 (Fan, Upadhye, & Worster, 2006). The selection of Findrisc for our protocol is, therefore, justified, since it is considered as the first step in the identification of previously unknown hyperglycaemia (Schwarz, Li, Lindstrom, & Tuomilehto, 2009), at least in Caucasian populations, which may be reasonable, considering that it is based on the most important established risk factors for this condition (e.g. age, BMI, family history).

The use of the highest EPB code alone to screen for previously unknown hyperglycaemia (Model II) had a low discriminating capacity (AUC=0.62), in spite of the fact that subjects with a worst periodontal condition (EPB codes 3-4) exhibited a higher risk for presenting poor glycaemic control (82.3% of the subjects with a point-of-care HbA1c ≥5.7% presented an EPB code 3-4). This low screening capacity, however, is similar to that reported by (Lalla et al., 2013) (AUC=0.60) using the percentage of teeth with at least one pocket ≥5 mm and the number of missing teeth, as measures of periodontal status, and to other models combining periodontal measures with BMI or WC (AUC ranging from 0.65 to 0.71) (Acharya et al., 2018; Holm et al., 2016). This low predictive capacity of periodontal parameters reflects that they should not be used as sole predictors, since it is generally considered that models with AUC<0.75 have no real clinical usefulness for

the screening of undiagnosed hyperglycaemia (Fan et al., 2006). Nevertheless, when we added the EPB data to the Findrisc questionnaire data (Model III), the performance of the Findrisc score significantly improved (AUC=0.88).

As expected, adding the point-of-care HbA1c significantly improved the screening capacity (AUC≈0.96). Although HPLC is still considered the reference method, several HbA1c point-of-care devices are available in the market, being less time-consuming (not requiring venous blood collection) and providing adequate screening capacity without the need for strict laboratory validation (Cagliero, Levina, & Nathan, 1999). In the case of the *DiabetRisk* study, we used the A1CNow$^{+®}$ device (Bayer Healthcare, Leverkusen, Germany), which has shown validation data that correlates with the HPLC reference results (Jiang et al., 2014).

The potential benefits of early detection and treatment of patients with diabetes, were reported for the first time in a multicentre randomized clinical trial (ADDITION-Europe) (Griffin et al., 2011), demonstrating that screening for diabetes, followed by intensive treatment, was associated with a significant reduction in the risk for cardiovascular morbidity and mortality (Herman et al., 2015). Due to the significant impact of hyperglycaemia on public health, existing healthcare facilities as dental offices or community pharmacies have been proposed as convenient places to screen for subjects at risk (Krass et al., 2017). The *DiabetRisk* study has used a model using minimally-invasive and cost-effective methods to screen for undiagnosed hyperglycaemia in the clinical setting. These initiatives may only be generalized in dental practices if they are easy to perform (and Findrisc consists mainly on self-reported information) and with minimum costs (such as the ones derived from the point-of-care HbA1c). Noteworthy, the reported predictive values are similar or even superior to those reported using other screening approaches in medical settings (Buijsse, Simmons, Griffin, & Schulze, 2011).

This study is not free of limitations. Although the use of a network of dental clinics may provide information generalized to the entire population looking for dental care, it has to be recognized that the periodontal data used in our analyses were recorded by multiple experienced examiners who did not perform any specific calibration exercise,

but received several training sessions. However, as previously stated, the objective of this study was to evaluate the actual outcomes of screening for diabetes in dental practices, as a field trial rather than a tightly calibrated environment (Genco et al., 2014). Another limitation may be related to those subjects that, in spite of being identified as subjects at risk of hyperglycaemia, did not return with a definitive diagnosis to the dental office (n=23, 14.5% of those referred to their physician).

In conclusion and in support of the recommendations by the IDF and other scientific associations (Sanz et al., 2018), the DiabetRisk study confirms the dentist´s role in the screening for unknown hyperglycaemia. The results of this study support the use of a protocol combining a validated questionnaire together with a point-of-care HbA1c, with or without a basic periodontal exam, as a useful tool to screen for subjects under risk of diabetes or prediabetes. The proposed screening protocol could be easily implemented in the dental office, as demonstrated by the positive feedback from the SEPA Research network of clinics involved in this study, although further long-term studies are needed to calculate the cost-effectiveness of this screening protocol and to confirm its usefulness in other populations.

Figure 1. Flow chart of patient inclusion and study design.

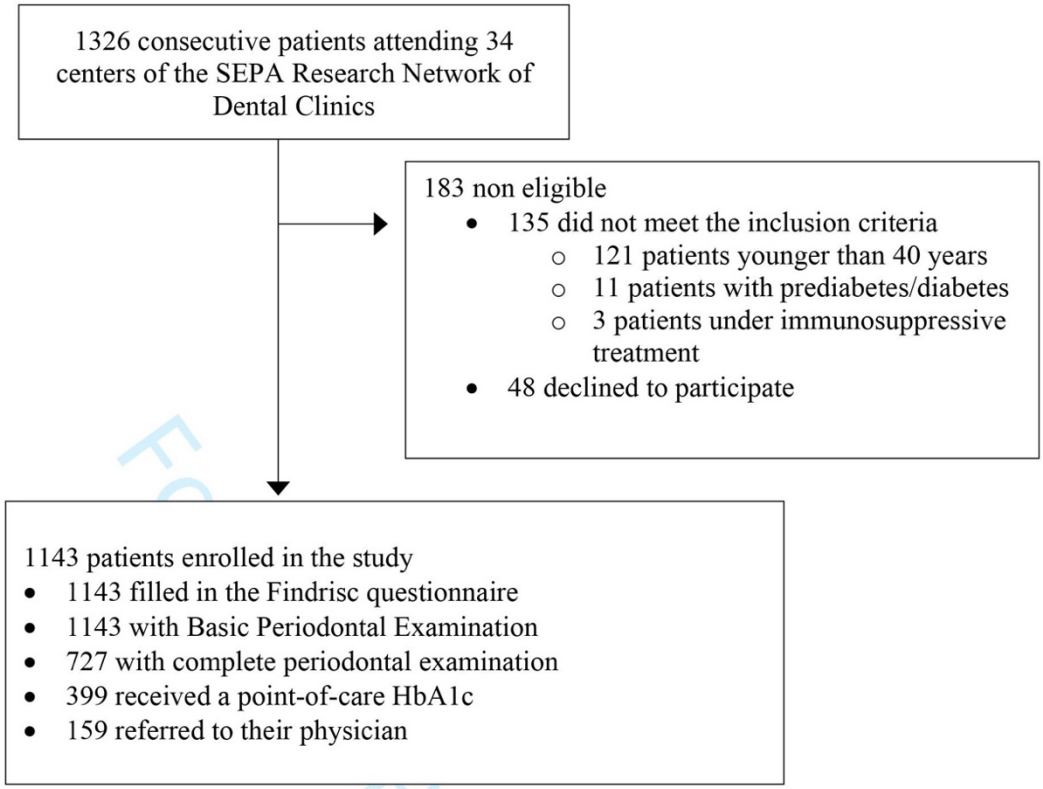
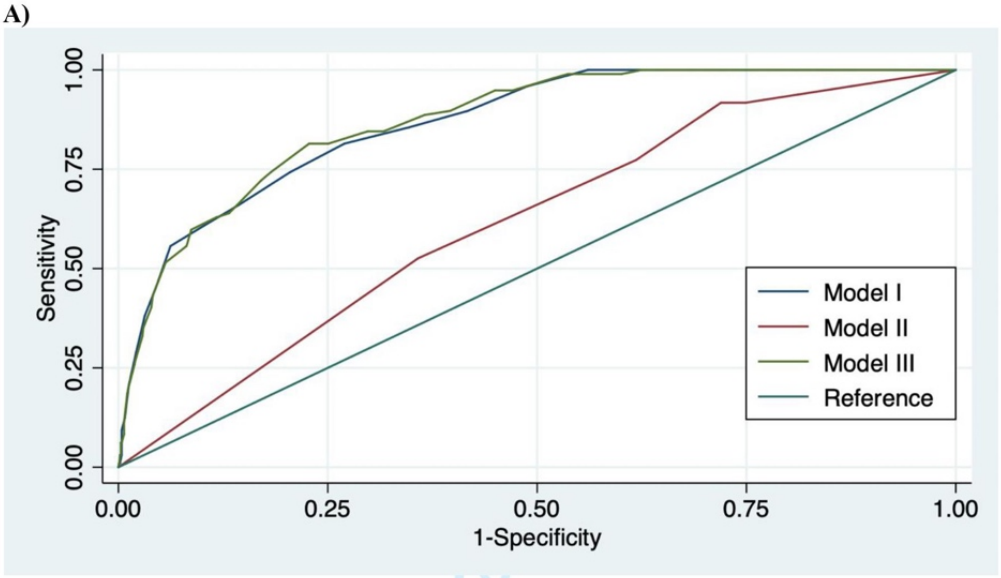


Figure 2. Receiver operating characteristic (ROC) curves for the various predictive models built by logistic regression:
A) Models I, II and III (n=1143). Model I: Findrisc score, area under the ROC curve (AUC) = 0.866 (95% confidence interval - CI [0.833; 0.900]); Model II: EPB (Basic Periodontal Exam) Code, AUC = 0.617 (95% CI [0.588; 0.645]); Model III: Findrisc score + EPB code, AUC = 0.876 (95% CI [0.845; 0.906]).
B) Models IV, V and VI (n=399). Model IV: Findrisc score + point-of-care HbA1c, AUC = 0.960 (95% CI [0.941; 0.979]); Model V: BPE code + point-of-care HbA1c, AUC = 0.958 (95% CI [0.937; 0.978]); Model VI: Findrisc score + EPB code + point-of-care HbA1c, AUC = 0.962 (95% CI [0.943; 0.981]).

A)



B)

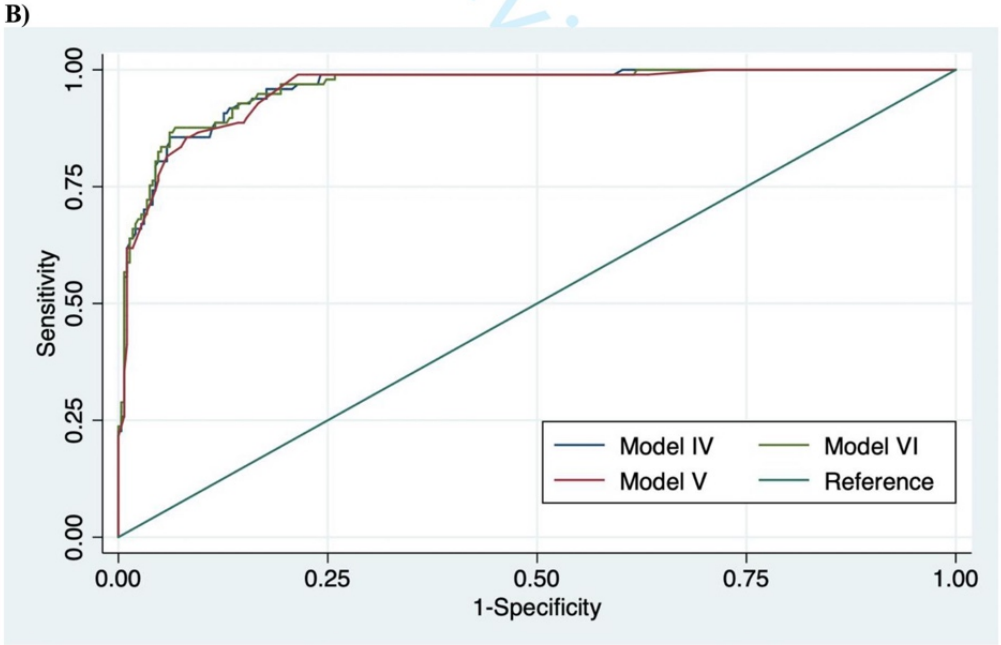


Table 1. Subjects characteristics and Findrisc components by confirmed glycemic status (N=1143)

	Metabolic Health	Prediabetes	Diabetes	Not Confirmed	Overall p- value
Number of participants (N)	1023 (89.5%)	69 (6.0%)	28 (2.5%)	23 (2.0%)	
Age (years)	54.3 (10.8)	58.1 (9.5)*	63.6 (10.4)*†	58.4 (8.0)	**<0.001**
Gender					0.796
Female	601 (58.8%)	39 (56.5%)	14 (50.0%)	14 (60.9%)	
Male	422 (41.3%)	30 (43.5%)	14 (50.0%)	9 (39.1%)	
Smoking status					0.613
Never/former smoker	783 (76.5%)	48 (69.6%)	21 (75.0%)	18 (78.3%)	
Current smoker	240 (23.5%)	21 (30.4%)	7 (25.0%)	5 (21.7%)	
Findrisc questionnaire score					**<0.001**
Low (0-6)	459 (44.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Slightly elevated (7-11)	370 (36.2%)	23 (33.3%)	2 (7.1%)	3 (13.0%)	
Moderate (12-14)	140 (13.7%)	12 (17.4%)	6 (21.4%)	9 (39.1%)	
High (15-20)	51 (5.0%)	28 (40.6%)	17 (60.7%)	10 (43.5)	
Very High (≥21)	3 (0.3%)	6 (8.7%)	3 (10.7%)	1 (4.4%)	
Body Mass Index (kg/m²)	24.9 (4.3)	28.3 (4.7)*	30.3 (4.9)*†‡	27.6 (3.9)*	**0.013**
Waist circumference (cm)					
Men					0.538
<94 cm	83 (27.3%)	6 (23.1%)	4 (23.5%)	2 (28.6%)	
94-102 cm	127 (41.8%)	8 (30.8%)	10 (58.8%)	3 (42.9.0%)	
>102 cm	94 (30.9%)	12 (46.2%)	3 (17.7%)	2 (28.6%)	
Women					**0.004**
<80 cm	170 (34.8%)	12 (30.0%)	0 (0.0%)	6 (40.0%)	
80-88 cm	169 (34.6%)	16 (40.0%)	1 (9.1%)	5 (33.3%)	
>88 cm	149 (31.5%)	12 (30.0%)	10 (90.9%)	4 (26.7%)	
Daily physical exercise					0.257
No	260 (32.5%)	14 (21.2%)	7 (25.9%)	7 (30.4%)	
Yes	539 (67.5%)	52 (78.8%)	20 (74.1%)	16 (69.6%)	
Vegetables or fruits consumption					0.428
Not every day	164 (20.4%)	8 (12.1%)	6 (22.2%)	5 (21.7%)	
Every day	640 (79.6%)	58 (87.9%)	21 (77.8%)	18 (78.3%)	
Current or past use of blood pressure medication					**0.012**
No	141 (17.5%)	19 (29.3%)	1 (3.7%)	2 (8.7%)	
Yes	666 (82.5%)	46 (70.8%)	26 (96.3%)	21 (91.3%)	
History of high blood glucose					0.202
No	736 (91.9%)	55 (84.6%)	26 (92.9%)	20 (87.0%)	
Yes	65 (8.1%)	10 (15.4%)	2 (7.1%)	3 (13.0%)	
Family history of diabetes					0.177
No	426 (53.2%)	34 (51.5%)	10 (35.7%)	14 (63.6%)	
Yes, second degree relative	145 (18.1%)	12 (18.2%)	9 (32.1%)	6 (27.3%)	
Yes, first degree relative	230 (28.7%)	20 (30.3%)	9 (32.1%)	2 (9.1%)	
Point-of-care HbA1c (%)	5.4 (0.4)	6.0 (0.3)*	6.9 (0.7)*†‡	5.8 (0.1)*	**<0.001**

Data are shown as mean (standard deviation, SD) or n (%).
Overall p values calculated by means of ANOVA F test or Chi-square test, comparing the four groups.
* Statistically significant difference compared with metabolic health.
† Statistically significant difference compared with prediabetes.
‡ Statistically significant difference compared with "not confirmed" category.
First degree relative: parent, brother, sister or own child; Second degree relative: grandparent, aunt, uncle or first cousin.

Table 2. Periodontal characteristics by confirmed glycemic status (N=1143)

	Metabolic Health (n=1023)	Prediabetes (n=69)	Diabetes (n=28)	Not Confirmed (n=23)	Overall p- value
Number of missing teeth	3.5 (4.4)	3.4 (5.3)	5.3 (3.1)	5.2 (4.3)	0.154
Highest Basic Periodontal Examination Code					**<0.001**
Code 0	262 (25.6%)	5 (7.3%)	3 (10.7%)	0 (0.0%)	
Code 1	31 (3.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Code 2	105 (10.3%)	9 (13.0%)	5 (17.9%)	1 (4.4%)	
Code 3	269 (26.3%)	16 (23.2%)	8 (28.6%)	3 (13.0%)	
Code 4	355 (34.7%)	39 (56.5%)	12 (42.9%)	19 (82.6%)	
Presence of plaque (%)	62.9% (31.2)	60.2% (28.8)	57.5% (26.7)	66.5% (27.6)	0.764
Bleeding on probing (%)	37.5% (25.1)	46.1 (31.6)	50.3% (27.5)	47.4% (28.9)	0.478
Mean probing depth (mm)	3.4 (1.2)	3.5 (1.0)	4.0 (0.9)*†	3.7 (0.7)	**0.035**
Mean clinical attachment level (mm)	4.1 (1.6)	4.2 (2.4)	4.8 (1.3)	4.5 (1.2)	0.266
Percentage of sites with PPD ≥4 mm	46.0% (28.8)	51.8% (27.0)	50.0% (33.3)	56.9% (27.9)	0.182
Percentage of sites with PPD >6 mm	4.0% (4.2)	7.0% (18.6)	16.4% (33.0)*†	7.5% (19.6)	0.096

Data are shown as mean (standard deviation, SD) or n (%).
Overall p values calculated by means of ANOVA F test or Chi-square test, comparing all 4 groups.
* Statistically significant difference compared with metabolic health.
† Statistically significant difference compared with prediabetes.
PPD, probing pocket depth.

Table 3. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for detection of hyperglycemia using optimal cut-off points. Optimal cut-off points are defined as those closer to the upper left angle of the receiver operating characteristic (ROC) curve (Sensitivity =1; Specificity =1).

	Sensitivity	Specificity	PPV	NPV
Model I				
Findrisc Score ≥11	0.81	0.73	0.22	0.98
Model II				
EPB Code 4	0.53	0.64	0.12	0.94
Model III				
Findrisc Score ≥11 or EPB Code 4	0.79	0.79	0.27	0.98
Point-of-care HbA1c				
Point-of-care HbA1c ≥5.8%	0.74	0.95	0.84	0.92
Model IV				
Findrisc Score ≥11 or point-of-care HbA1c ≥5.8%	0.86	0.94	0.81	0.95
Model V				
EPB Code 4 or point-of-care HbA1c ≥5.8%	0.87	0.91	0.75	0.95
Model VI				
Findrisc Score ≥11 or EPB Code 4 or point-of-care HbA1c ≥5.8%	0.88	0.93	0.81	0.96

EPB: *Examen Periodontal Básico* (Basic Periodontal Examination).

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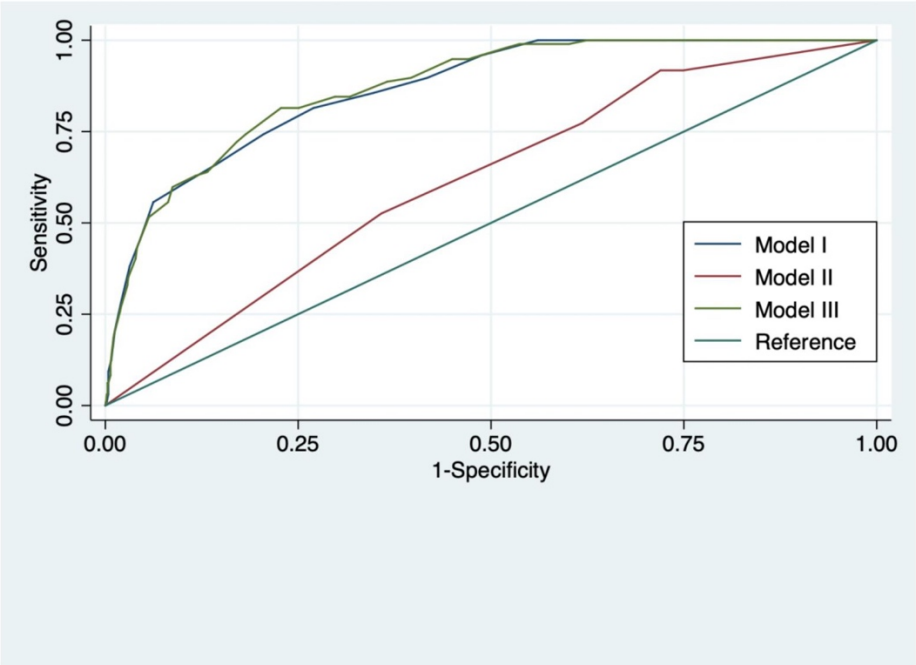

Figure 2. Receiver operating characteristic (ROC) curves for the various predictive models built by logistic regression.
A) Models I, II and III (N=1143). Model I: Findrisc score, area under the ROC curve (AUC) = 0.866 (95% confidence interval (CI) [0.833; 0.900]); Model II: EPB (Basic Periodontal Exam) Code, AUC = 0.617 (95% CI [0.588; 0.645]); Model III: Findrisc score + EPB code, AUC = 0.876 (95% CI [0.845; 0.906]).

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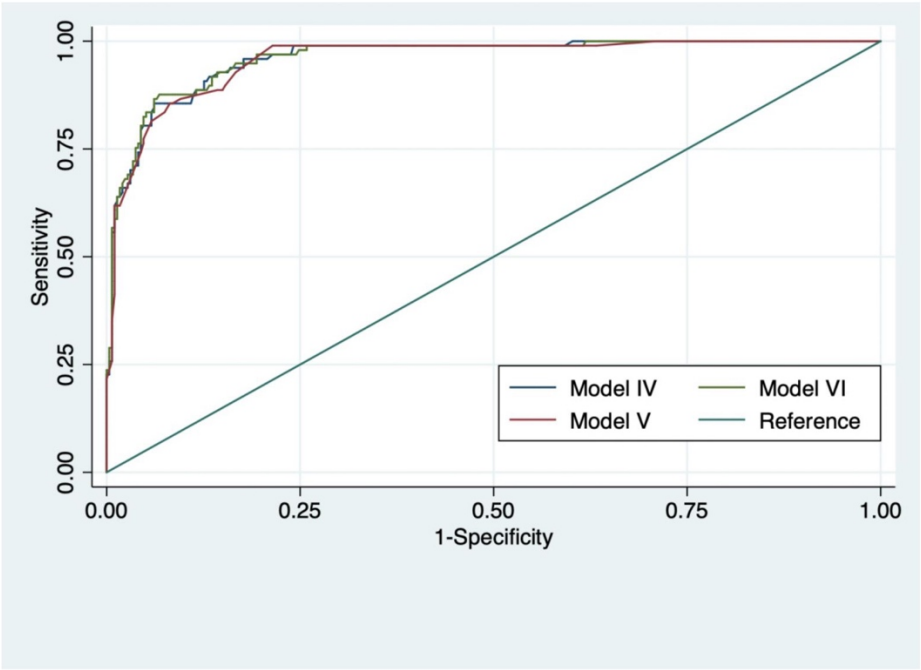

Figure 2. Receiver operating characteristic (ROC) curves for the various predictive models built by logistic regression.B) Models IV, V and VI (n=399). Model IV: Findrisc score + point-of-care HbA1c, AUC = 0.960 (95% CI [0.941; 0.979]); Model V: BPE code + point-of-care HbA1c, AUC = 0.958 (95% CI [0.937; 0.978]); Model VI: Findrisc score + EPB code + point-of-care HbA1c, AUC = 0.962 (95% CI [0.943; 0.981]).

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VII. DISCUSSION

The global objective of the present doctoral thesis was to evaluate the association between periodontitis and two metabolic conditions (hyperglycaemia and MetS), and to determine whether periodontal therapy or screening tools, both for periodontitis or hyperglycaemia, might be useful in the simultaneous management and prevention of periodontitis and metabolic diseases.

Specifically, an oral epidemiological survey on a representative sample of the Spanish employed population (WORALTH Study) was used to evaluate the association between periodontal status, as determined by the Community Periodontal Index (CPI) and Clinical Attachment Level (CAL), and i) hyperglycaemia (either prediabetes or DM) or ii) MetS (according to IDF and NCEP-ATP III criteria). After adjusting for potential confounders (i.e. age, gender, occupation, education, smoking, BMI, waist circumference, triglycerides, total cholesterol and systolic/diastolic blood pressure), DM was found to be significantly associated with severe periodontitis, as defined by CPI code 4 (OR=1.86; 95% CI 1.13-3.07); however, prediabetes was not significantly associated with periodontal status **(Study #1; (Montero, Carasol, et al., 2019))**. In the case of MetS, and regardless of the definition, it was found to be significantly associated with both CPI code 4 (OR=1.41; 95% CI 1.10-1.81 for the IDF definition) and CAL ≥6 mm (OR=1.37; 95% 1.04-1.82 for the IDF definition), with hypertension being the component with a strongest association with periodontal status, and the association being stronger among women **(Study #2*;* Montero et al. 2020. *Accepted for publication*)**. The results of these two epidemiological studies clearly demonstrate that there is an association between periodontitis and certain metabolic disorders in the Spanish employed population, which makes the case for the promotion of oral health education and the performance of regular periodontal check-ups in subjects with DM or MetS.

To evaluate the effect of periodontal treatment on systemic inflammation in patients with MetS, a RCT was carried out. The hypothesis was that effective treatment to reduce periodontal inflammation may lead to a reduction in cardiovascular risk in these patients. The results confirmed this hypothesis, as the IPT group experienced a

statistically significant reduction in hs-CRP after 6 months (adjusted difference between treatment groups was 1.2 mg/L; p=0.004), together with a significant improvement in the vascular function (as determined by blood pressure measurements) ***(Study #3; (Montero et al., 2020))***. These findings support the evidence that effective periodontal therapy has a positive short-term systemic effect in patients with MetS, although studies with a longer follow-up times are needed to confirm whether these benefits could be sustained over time, and whether they lead to reductions in cardiovascular morbidity and mortality.

The last part of the present work investigates the potential benefits of carrying out integrative approaches by physicians and periodontists in the management of metabolic disorders and periodontitis. With that objective in mind, a predictive model to screen for moderate-to-severe periodontitis was established using a combination of cardio-metabolic and socio-demographic variables from NHANES 2011-2012 data. The proposed model comprising just five variables, commonly registered in medical care settings (i.e. age, gender, ethnicity, smoking habit and HbA1c) presented a sensitivity of 70% and a specificity of 68% to identify patients with moderate-to-severe periodontitis, making it a good candidate to be regularly used by physicians in primary care settings ***(Study #4; (Montero, Herrera, et al., 2019)).*** However, further validation of the model in other, if possible, prospective cohorts, is mandatory before it can be included in formal clinical guidelines.

Analogous to the screening for periodontitis by the medical community, the dental clinic has been proposed as an ideal setting for the detection of previously unknown diabetes or prediabetes. With that objective in mind, a research project, developed as a collaboration between the Spanish Society of Periodontology (SEPA) and the Spanish Society of Diabetes (SED) through the establishment of a joint Working Group, resulted into a research protocol within the Research Network of Dental Clinics coordinated by SEPA. The protocol aimed to evaluate different screening protocols for risk assessment in the detection of undiagnosed hyperglycaemia in a network of dental clinics (including both dental schools and private practices). The results demonstrated that the dental setting may be a suitable place for the identification of patients with previously

unknown hyperglycaemia, as 8.5% of the included subjects were diagnosed either with diabetes or prediabetes. However, the periodontal assessment did not seem to provide any further effectiveness over the use of an already validated self-reported questionnaire (FINDRISC and a point-of-care HbA1c test, that presented a sensitivity of 86% and a specificity of 94% for the detection of hyperglycaemia ***(Study #5, Montero et al. 2020. Accepted for publication)***. The results of Study #5 support the recommendations by the International Diabetes Federation (IDF) and the European Federation of Periodontology (EFP) regarding the dentist´s role in the screening for undiagnosed hyperglycaemia (Sanz et al., 2018), and the proposed model seems to be easy to implement in the dental clinic.

Epidemiological association between periodontal status and cardiometabolic risk factors

The cross-sectional association between type 2 DM and periodontitis has been already substantiated. The studies performed on the Pima Indians of Arizona, the population with the highest reported prevalence of DM in the world (40-50% in adults over 35 years of age) (Knowler, Bennett, Hamman, & Miller, 1978), clearly demonstrated a strong correlation between DM and the prevalence and severity of periodontitis, independently of already known risk factors/determinants such as age or oral hygiene (Emrich, Shlossman, & Genco, 1991). Subsequently, similar findings were reported in other populations, such as subjects with type 1 DM or Gullah- African Americans (Fernandes et al., 2009; Hodge et al., 2012). Nevertheless, the definitive causative two-way relationship between DM and periodontitis emanates from longitudinal trials establishing that: i) DM and poor glycaemic control correlates with incident periodontitis and more severe progression of the disease (Bandyopadhyay, Marlow, Fernandes, & Leite, 2010; Nelson et al., 1990); and ii) periodontitis affects glycaemic control in diabetic (Chang et al., 2017; Taylor et al., 1996) and not diabetic subjects (Graziani et al., 2018).

However, the potential association between periodontitis and prediabetes is less clear. This association could be of particular interest, since prediabetes is, in itself, a risk factor for the development of cardiovascular, renal and neurologic complications, but at the

same time is a reversible condition (Fonseca, 2009; Shaye et al., 2012; Tabak et al., 2012). If periodontitis and prediabetes are significantly associated, it could be hypothesized that periodontal treatment may reduce progression to hyperglycaemia in these patients. Indeed, a secondary analysis of a multicentre RCT (ABPARO, "Antiobiotika und Periodontitis) observed a decrease in the number of prediabetes patients to approximately 50%, after 27.5 months, when compared to baseline (Kocher et al., 2018). Unfortunately, since the main objective of the trial was not to evaluate the impact of treatment on HbA1c, no randomization across groups stratified by baseline HbA1c was performed. Therefore, the possibility that changes in HbA1 were attributable to regression to the mean, limits conclusions from this post-hoc analysis to those associated with hypothesis generation.

In the WORALTH study, DM was significantly associated with severe periodontitis, as defined by CPI code 4 (OR=1.86; 95% CI 1.13-3.07). Conversely, no association could be found between periodontitis (assessed by CPI or CAL) and prediabetes (as defined by IFG) among the 4,222 subjects receiving a periodontal examination after adjusting for confounders **(Study #1; (Montero, Carasol, et al., 2019))**. Results of this investigation are in agreement with other studies using different periodontal indices or periodontitis case definitions. Two studies performed in Germany failed to find any association between different measures of periodontitis (mean probing depth, percentage of sites with CAL≥ 4 mm) and prediabetes (Kowall et al., 2015; Noack et al., 2000). Similarly, data derived from NHANES 2019-2012 do not support the proposal that periodontitis, as defined by the Centres for Disease Control/American Academy of Periodontology (CDC/AAP) case definitions, was more likely in people with IFG (Arora et al., 2014; Eke et al., 2016). Noteworthy, significant associations between periodontitis and prediabetes were observed only when either periodontal outcomes (mostly CAL) or IFG were considered as continuous variables, with subjects in the upper quintile category of CAL or those with IFG=111-125 mg/dL presenting an association between both diseases (Y. H. Choi et al., 2011; Hong, Noh, & Kim, 2016). Thus, it seems that is the level of hyperglycaemia, rather than the diagnosis of prediabetes/DM, is the likely determining factor in the association of periodontitis and abnormal glucose regulation. It is important to understand that the cut-off values used in the diagnosis of DM are based on the risk

of developing diabetes-related complications (particularly, retinopathies). Unfortunately, little attention has been paid to periodontitis, despite being related to insulin resistance, so it is possible that periodontitis could predict the incidence of DM.

Early insulin resistance and β-cells function, as assessed by HOMA-IR and HOMA-IS have demonstrated to be associated with the development of periodontal pockets and periodontal breakdown, suggesting that early hyperglycemia, before becoming overt DM, may influence the pathogenesis of periodontitis (Allen et al., 2011; P. Timonen et al., 2013). Particularly interesting is a community-based cohort study of 5,885 Taiwanese that explored the temporal sequence of the bidirectional relationship between periodontitis and hyperglycaemia (Chiu et al., 2015). In that study, it was reported that incident cases of periodontitis (as defined as CPI≥ 3) presented a 33% increase risk for incident hyperglycaemia (prediabetes and DM), while at the same time prediabetes and DM led to a significantly higher risk for periodontitis [hazard ratio (HR)=1.25 for prediabetes and HR=1.95 for DM].

In this study, results were heavily influenced by the low prevalence of DM in the sample (2.24%), which is lower than the prevalence reported in other population-based studies, such as NHANES 2009-2012 in the USA (12.6%) or SHIP-Trend in Germany (11.8%) (Eke et al., 2016; Kowall et al., 2015). The WORALTH study was restricted to the employed population (range 16-65 years), which may account for this low prevalence of DM, since this disease prevalence significantly increases with age. The low number of diabetes cases observed in the WORALTH study may be related with the reported differences by sex and age, with periodontitis being significantly associated with DM in men, but not in women (there were just 7 females with DM). In terms of age, this study reported a stronger association between DM and CPI =4 in adults <45 years, similarly to what it has been reported for the association between atherosclerotic cardiovascular disease and periodontitis (Dietrich, Sharma, Walter, Weston, & Beck, 2013), suggesting a possible higher aggressiveness of the chronic inflammation secondary to periodontitis or diabetes in young individuals.

In conclusion, even if no specific association was observed in the WORALTH study between periodontitis and prediabetes, the clear association observed for DM, together with the overwhelming scientific literature, support a linear bidirectional relationship between insulin resistance/hyperglycaemia and periodontitis. However, the glycemic control threshold over which periodontitis risk can be increased is unknown, and the same is true for the severity of periodontitis that contributes to hyperglycaemia. Longitudinal studies using different measures for abnormal glucose regulation (HOMA-IR, HOMA-β, fasting plasma glucose, oral glucose tolerance) and measures to adequately define the prevalence and extent of periodontitis (CAL and probing depth at site-, tooth- and patient-level according to specific thresholds) are needed in order to clarify the relationship between these conditions.

The WORALTH Study was also used to investigate the association between periodontitis and MetS **(Study #2, Montero et al. 2020. *Accepted for publication*)**. Previous studies, including a systematic review with meta-analysis, have found significant associations between periodontitis and MetS (Nibali et al., 2013). However, this association still remains controversial, since the diagnostic criteria used to define the case of MetS and the case definition of periodontitis vary between studies, and some studies were only capable of finding associations in specific groups, such as women, diabetic patients or in specific age groups (D'Aiuto et al., 2008; Furuta et al., 2013; Sora et al., 2013; Y. K. Tu et al., 2013), while others observed no association between MetS and periodontitis at all (Benguigui et al., 2010; LaMonte et al., 2014; Zuk et al., 2017). Hence, there was a need of further research to clarify these inconsistencies and heterogeneous results among distinct groups, and to evaluate the association between the individual components of MetS and periodontitis, especially in Western populations, since published results on those populations are particularly conflicting.

Our study, included 4,353 patients in the final analyses, and it was reported that severe periodontitis (either defined as CPI code 4, or CAL ≥ 6 mm), was consistently associated with MetS, independently of its definition. Specifically, ORs for the association between

CPI code 4 and MetS were 1.41 and 1.58 for the IDF and NCEP-ATP III definitions, respectively; while the associations between CAL ≥ 6 mm and MetS were 1.37 and 1.57. Furthermore, despite the fact that not all the components of the syndrome presented a significant association with severe periodontitis in fully adjusted models, there was a tendency towards a linear relationship between a poorer periodontal condition and a higher prevalence of cardiometabolic abnormalities. As an example, the prevalence of hypertension in patients with severe periodontitis (CPI code 4 or CAL≥ 6 mm) was double than the prevalence among subjects with CPI code 0 or CAL= 0-3 mm, and similar observations were found for abdominal obesity (defined by waist circumference), hyperglycaemia or dyslipidaemia.

In agreement with previous reports (D'Aiuto et al., 2008; Furuta et al., 2013; Y.-K. Tu, D'Aiuto, Lin, Chen, & Chien, 2013; Y. K. Tu et al., 2013), in our cross-sectional study, the association between periodontitis and MetS was stronger in women. A significant association between severe periodontitis and MetS among males was just observed for the NCEP-ATP III definition and CPI code 4 (OR=1.44; 95% CI 1.01-2.04). These findings may have different explanations. It is expected that periodontal infections may have a weaker association than other major environmental risk factors with MetS (e.g. tobacco smoking). The fact that men tend to have a less healthy lifestyle may “mask” the contribution of periodontitis to MetS, explaining the smaller ORs. Another possible explanation is that, among patients with MetS, females present higher levels of CRP than men (Saltevo, Vanhala, Kautiainen, Kumpusalo, & Laakso, 2008), suggesting that the higher levels of systemic inflammation could explain the stronger association with periodontitis. Lastly, sex hormones may play an important role in the pathogenesis of both diseases: while inflammation decreases oestradiol production and thus, reduce the protective effect of oestrogens on body fat distribution and insulin action (Alpizar & Spicer, 1994), oestrogens alter the host response to the subgingival biofilm (Mealey & Moritz, 2003), contributing to periodontal diseases in females. Therefore, gender differences may influence preventive and therapeutic measures for patients with periodontitis and MetS.

Among the individual components of MetS, hypertension presented with the strongest association with periodontal outcomes (OR= 1.94; 95% CI 1.49-2.53; for the association between CAL≥ 6 mm and MetS according to the IDF definition). A recent systematic review with meta-analysis showed an OR=1.49 (95% CI 1.09-2.50) for the association between severe periodontitis and high BP (Muñoz Aguilera et al., 2020). Moreover, in that systematic review, a reduction in BP following periodontal treatment was observed, with reductions in systolic blood pressure (SBP) ranging from 3 to 12.5 mmHg and in diastolic blood pressure (DBP) from 0 to 10 mmHg. These results are in agreement with the ones reported in the third study of the present work **(Study #3; (Montero et al., 2020)**, since reductions of approximately 7 mmHg in SBP and DBP were observed in MetS patients with severe periodontitis after non-surgical periodontal therapy.

Surprisingly, in **Study #2,** no association could be found between abdominal obesity, as determined by waist circumference and periodontitis. Whilst several cross-sectional studies on MetS patients report either no association between obesity and periodontitis (Alhabashneh, Khader, Herra, Asa'ad, & Assad, 2015; Benguigui et al., 2010; D'Aiuto et al., 2008), or associations of small magnitude and not statistically significant (P Timonen et al., 2010), a recent meta-review including evidence from 14 systematic reviews suggested a positive association between obesity and periodontitis onset, progression, and response to periodontal treatment (Jean E Suvan, Finer, & D'Aiuto, 2018). Notably, in most of the studies included in the published systematic reviews on the association between obesity and periodontitis (Chaffee & Weston, 2010; Keller, Rohde, Raymond, & Heitmann, 2015; J. Suvan et al., 2011), data on glycemic status, lipid profiles or BP were not evaluated or not considered as potential confounders in the association. However, most of the studies specifically including MetS patients, elaborated multivariable regression models considering the majority of potential confounders. Therefore, it is possible that the strongest association with other individual components of the MetS (e.g., hyperglycemia, hypertension) leads to smaller or even non-significant ORs between periodontitis and obesity in this subset of patients. Indeed, when patients in the WORALTH Study were categorized by body size phenotype, obesity alone, with no concomitant cardiometabolic abnormalities (metabolically healthy obese, MHO), did not show association with periodontitis, while subjects

presenting altered metabolic profiles showed a greater tendency towards a worse periodontal condition, independently of being obese or with normal weight. These observations suggest the possible role of the low-grade systemic inflammatory status reported in severe periodontitis as a contributor in the pathophysiology of certain metabolic alterations, such as the increase in plasma glucose levels or abnormalities in the lipid profiles, even in the absence of large fat deposits. In any case, it should be noted that all anthropometric measures (WC, BMI, etc.) are not necessarily indicative of the amount of visceral fat, and therefore, in order to evaluate the potential association between adiposity and periodontitis, imaging techniques, such as magnetic resonance, should be used in the future.

Several limitations of the WORALTH Study should be acknowledged. Firstly, the cross-sectional nature of the study does not allow to ascertain any causal relationship. Secondly, the population was limited to employed subjects, not including older adults or children or adolescents, which could explain the relatively low prevalence of hyperglycaemia and MetS observed in this sample. Lastly, the most important limitations are related to the periodontal and medical examinations. On one hand, the periodontal exam was based on the WHO recommendations, consisting on a partial mouth recording that may lead to underestimation of the disease presence, severity and extent (Kingman, Susin, & Albandar, 2008; Susin, Kingman, & Albandar, 2005). What is more, CPI is not a diagnostic tool, but only a screening tool, same as other indices developed to evaluate periodontal treatment needs (e.g. Basic Periodontal Examination (Tugnait, Clerehugh, & Hirschmann, 2004)), and therefore, may hamper the accuracy of periodontal diagnosis, as it is based solely on the pocket component. However, a strict measure of attachment loss (i.e. CAL) was the other periodontal outcome variable registered in this survey. On the other hand, hyperglycaemia was assessed by fasting glucose only. Other parameters such as HOMA-IR, HOMA-β, oral glucose tolerance tests or HbA1c could have provided deeper information on insulin resistance, β-cell function and natural history of hyperglycemia in these patients, including long-term metabolic control. Similarly, anthropometric measures for the evaluation of central obesity could have been complemented with imaging techniques such as magnetic resonance or computed tomography, in order to properly evaluate adiposity and visceral fat.

Impact of periodontal therapy on systemic markers of inflammation in patients with MetS

The second part of the present doctoral thesis has evaluated, through a parallel-arm, double-blind, RCT, the impact of non-surgical (steps 1 and 2) periodontal treatment on systemic markers of inflammation in patients with MetS and periodontitis **(Study 3; (Montero et al., 2020)**. In this study, patients were randomly assigned to receive intensive periodontal treatment (IPT; consisting on scaling and root planing plus azithromycin 500 mg every day for 3 days) or minimal periodontal treatment (MPT; consisting on supragingival professional mechanical plaque removal plus a placebo), the outcomes assessed at baseline and 3 and 6 months after treatment were: markers of systemic inflammation and prothrombotic states (i.e. hs-CRP, α-1 antitrypsin and fibrinogen), pro-inflammatory cytokines (i.e. IL-1β, IL-6, IL-8 and TNF-α), indicators od carbohydrate and lipid metabolism, as well as BP. The results of the trial have shown that effective periodontal therapy in patients with severe periodontitis and MetS results in significant reductions of several cardiovascular risk biomarkers, such as hs-CRP, IL-1β, TNF-α, HbA1c and improved vascular function. Subjects in the IPT group experienced a 30.8% reduction in hs-CRP from baseline, resulting in an adjusted difference of 1.2 mg/L at 6 months, when compared to the MPT group. These findings support the contribution of periodontitis to systemic inflammation (Paraskevas, Huizinga, & Loos, 2008), particularly in patients with MetS, a condition identified as an important risk factor for cardiovascular disease (Mottillo et al., 2010).

One of the main characteristics of the study is related to the severity of both periodontal and systemic involvement of the enrolled subjects. More than 50% of the patients presented hs-CRP levels ≥3 mg/L at baseline, more than 25% presented HbA1c ≥ 7%, and mean BMI was close to class 3 obesity (≈38.0 kg/m^2). Despite being under specific cardioprotective drug therapies, such as calcium channel blockers, Angiotensin Converting Enzyme (ACE) inhibitors or statins, the patients included presented high levels of hs-CRP and an increased cardiovascular risk. Additionally, these patients presented with particularly severe forms of periodontitis (stages III/IV), with a strong infectious-inflammatory component, as represented by high percentages of periodontal

inflammation (bleeding on probing, BOP ≈60% in both groups) and presence of deep pockets (≈60% of sites with probing pocket depth, PPD ≥4 mm). Our results are of particular interest, since they provide the evidence that improving periodontal health may offer an important therapeutic opportunity to reduce the cardiovascular risk in patients with severe periodontitis and presenting difficulties in achieving cardiovascular stability. Importantly, the magnitude of the effect in the reduction of hs-CRP is within the range of extensively evaluated LDL-lowering therapies (Kinlay, 2007; Ridker et al., 2005).

A strength of the study was the statistically significant improvement in clinical and microbiological outcomes observed in the IPT, when compared with the MPT. One of the main criticisms of the results of two multicentre RCTs, that failed to report an any effect of non-surgical periodontal therapy on hs-CRP and HbA1c, in patients with recent cardiovascular events and diabetes, respectively, was that periodontal therapy did not achieve accepted standards of success in terms of residual periodontal inflammation (BOP) and dental biofilm (plaque) control (S. P. Engebretson et al., 2013; Offenbacher et al., 2009). In our study, there was a 43.7% reduction in the number of periodontal pockets ≥ 4 mm and a 37.6% reduction in the BOP score in the IPT group, while in PAVE (Offenbacher et al., 2009) the reduction in the extent of sites with PD ≥4 mm was 6.6%, and BOP was 38.3% at 6 months. At the end of the follow-up, in the PAVE trial, many of the test patients still had sufficient gingival inflammation to meet the periodontal entry criteria to be enrolled in the study for periodontal treatment.

Furthermore, in our study the tested intervention significantly reduced both the total counts of anaerobic bacteria and *P. gingivalis*. Although previous cross-sectional studies have shown an independent significant association between the presence of a significant subgingival bacterial burden and levels of periodontal pathogens, with surrogate measurements of atherosclerotic risk, such as intima-media thickness or hs-CRP (Desvarieux et al., 2005), this is the first RCT to show that effective periodontal treatment significantly reduced these microbiological exposure measurements and that this microbiological impact was associated with significant reductions in hs-CRP.

The impact of periodontal therapy was not limited to hs-CRP reductions, since several inflammatory cytokines, such as IL-1β or TNF-α, showed significant reductions at 3 months but not at the end of the follow-up. These results are somewhat in agreement with a recent RCT in periodontitis patients with type 2 DM, where significant differences between groups were noted in TNF-α after treatment (D´Aiuto et al., 2018). This finding may be meaningful, as both IL-1β and TNF-α have a pro-atherogenic effect and they have been established as significant and independent predictors of cardiovascular events and overall mortality (Tuomisto, Jousilahti, Sundvall, Pajunen, & Salomaa, 2006). However, there is conflicting evidence on the effect of periodontal therapy on these cytokines (D´Aiuto, Orlandi, & Gunsolley, 2013), and studying the effect of periodontal therapy on isolated inflammatory mediators does not necessarily provide an additional understanding on the links between periodontitis and systemic inflammation (Gaudilliere et al., 2019).

Additional statistically significant improvements in secondary outcomes, such as HbA1c and BP, were observed in this study. Periodontal inflammation has been identified as a driver to both insulin resistance and endothelial dysfunction, two of the most important risk factors in the pathogenesis of atherosclerotic CVD (Demmer et al., 2010; Teeuw et al., 2014; Tonetti et al., 2007). In our trial, the IPT group led a to a significant 0.3% reduction in HbA1c at 3 months, with differences between groups not reaching statistical significance at 6 months. Our results are in agreement with a 2015 Cochrane systematic review, concluding that the improvement in glucose control after periodontal therapy disappears after 3 months (Simpson et al., 2015). On the contrary, at least three other trials have reported positive effects of periodontal therapy on glycemic control in the long term (Chen et al., 2012; D´Aiuto et al., 2018; Koromantzos et al., 2011). The absence of a long-lasting effect in our study might be explained by the fact that, while aforementioned trials performed repeated subgingival instrumentations, or at least supragingival prophylaxis, every 3 months, no further periodontal therapy was provided in our study. This finding reinforces the concept that supportive periodontal care should be performed at regular intervals in order to decrease the intraoral bacterial burden and its systemic impact.

Recent evidence suggests that periodontitis may be associated with hypertension and that periodontal treatment could reduce arterial BP (Muñoz Aguilera et al., 2020; Vidal, Cordovil, Figueredo, & Fischer, 2013; Q.-B. Zhou et al., 2017). As occurs with hs-CRP levels, despite available treatments, hypertension remains poorly controlled in over half of hypertensive adults (Joffres et al., 2013). In our study, we observed an adjusted statistically significant difference of systolic BP of 7.8 mmHg, and of diastolic BP of 7.3 mmHg, at 3 months after IPT. The difference in diastolic BP persisted up to 6 months (11.0 mmHg). A 10 mmHg reduction in systolic BP, or a 5 mmHg reduction in diastolic BP, have been quantified to account for a 25-30% reduction of cardiovascular events (Law, Morris, & Wald, 2009), indicating that periodontal therapy may offer a novel alternative to pharmacological therapy to help in the management of hypertension.

This clinical trial, however, presents several limitations. One limitation is that our findings could be partly attributed to the effect of azithromycin by itself. Indeed, results from the ACADEMIC (Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia) study showed that this systemic antimicrobial could lead to a significant reduction in a global inflammatory score comprised by hs-CRP, IL-1β, IL-6 and TNF-α, at 6 months (Anderson et al., 1999). However, the treatment protocol in the ACADEMIC study consisted on azithromycin 500 mg/d for 3 days followed by 500 mg/weekly for 3 months, so administration was repeated for a longer period of time. In addition, no specific effect was observed for hs-CRP at 3 or 6 months- when it was considered alone, and not as part of the global inflammatory score. Nevertheless, the periodontitis cases included in our trial are routinely treated in everyday practice by a combination of both mechanical and antimicrobial therapy due to the severity of the infectious-inflammatory component (Herrera, Alonso, Leon, Roldan, & Sanz, 2008), so the IPT group consisted of the usual specialist care for these forms of disease. Another limitation is that no periodontal supportive care was provided in this study. Probably, the short-term follow-up (6 months), prevented the observation of certain recurrence of the periodontal infection, that may hamper the lower state of systemic inflammation obtained after therapy. Future studies should address the efficacy of the entire course of periodontal therapy (non-surgical or steps 1 and 2, surgical or step 3 -if needed- and supportive periodontal care) on patients with high cardiovascular risk in the long term.

Lastly, the selection of the main outcome variable (hs-CRP) may present several shortcomings: although it is the inflammatory biomarker most frequently reported for use in screening and cardiovascular risk reclassification, it is questionable whether its incremental predictive value in risk prediction over the classical Framingham risk factors is clinically meaningful, or useful to tailor statin therapy (Yousuf et al., 2013). Further investigations on the effect of periodontal therapy on the reduction of CVD risk should also focus on other surrogate measures of subclinical atherosclerosis, like the coronary artery calcium (CAC) or, when possible, in true endpoints such as major cardiovascular events.

Integrative approaches for the screening of periodontitis and hyperglycemia

The third part of the present doctoral thesis focussed on screening strategies that could be applied in primary health care centers or in the dental office, in light of the association between periodontitis, diabetes and cardiovascular diseases. Therefore, a first attempt was made to investigate the associations between well-established cardio-metabolic risk measures (i.e. age, gender, smoking habit, BMI, blood pressure, cholesterol and HbA1c) and moderate-to-severe periodontitis using the NHANES 2011-2012 dataset, and subsequently, to develop and validate a predictive model for moderate-to-severe periodontitis using a combination of cardio-metabolic and socio-demographic variables **(Study #4; (Montero, Herrera, et al., 2019))**.

After excluding those participants aged ≤30 years, with ≤14 teeth or without periodontal examination or registered data for age, gender, ethnicity, smoking habit, BMI, BP, total cholesterol or HbA1c, 3,017 subjects were finally included in the analyses. Prevalence of moderate and severe periodontitis were 37% and 13%, respectively, which are similar estimates to those reported by Eke et al. when combining NHANES 2019 to 2012 data (Eke et al., 2015). In agreement with previous findings from NHANES, smoking habit, followed by age, gender and HbA1c were the strongest indicators for moderate-to-severe periodontitis (according to the AAP-CDC case definition) and to mean CAL as continuous measure (Albandar, Brunelle, & Kingman, 1999; Eke, Dye, Wei, Thornton-Evans, & Genco, 2012; Tomar & Asma, 2000).

Similarly to the results from Study #2, BMI was not a significant predictor for periodontitis after adjusting for confounders. However, in Study #4, a statistically significant association was observed for obese subjects <50 years, suggesting that the association between obesity and periodontitis may be stronger in younger individuals, which is in agreement with a previous systematic review with meta-analysis (Chaffee & Weston, 2010). As previously stated, BMI and other anthropometric measures do not allow any distinction between visceral and subcutaneous adiposity, former being the main source of pro-inflammatory adipokines (Fox et al., 2007), and for this reason, these associations should be interpreted with caution.

Finally, a simplified predictive model for moderate-to-severe periodontitis with five variables (age, gender, ethnicity, HbA1c and smoking habit) was proposed and validated by means of a bootstrap validation approach. The model presented an Area Under the Curve (AUC) of 0.773, with sensitivity and specificity values of 70.0% and 67.6%, respectively. This model, together with the prognostic index table presenting the relative risks (RRs) for all possible combinations of the predictive variables, could be useful as a screening tool for moderate-to-severe periodontitis by healthcare professionals, for example, in primary healthcare centres. Future research should focus on the potential of this algorithm to be included in electronic health records to easily help physicians to identify subjects at risk of suffering moderate-to-severe periodontitis. Unfortunately, the predictive model is only applicable to the USA population, and before extrapolating it to countries with different demographics and health-care systems, it would need validation in other, if possible, prospective cohort studies. Furthermore, the model was built with the NHANES 2011-2012 dataset, and probably, it would need to be updated with more recent available data (the last NHANES survey with a full-mouth periodontal examination corresponds to 2013-2014), even if no significant changes in prevalence or risk indicators for periodontitis are expected (Eke, Borgnakke, & Genco, 2020).

Thus, the proposed model should be considered as a proof of principle that highlights the bidirectional relationship between periodontitis and cardiometabolic health, demonstrating how different integrative measures may be applied not only in the dental

setting, but also in other environments to allow for early identification, and eventually, treatment, that may be helpful in the co-management of periodontal diseases and hyperglycaemia (i.e. prediabetes and DM).

It has been proposed that the dental setting may also be a suitable environment for diabetes screening (Lalla, Cheng, Kunzel, Burkett, & Lamster, 2013; Lalla, Kunzel, Burkett, Cheng, & Lamster, 2011). With that objective in mind, a field trial (DiabetRisk Study) was designed as an initiative of the joint Working Group "Diabetes and Periodontal Diseases" of the Spanish Society of Diabetes (SED) and the Spanish Society of Periodontology (SEPA) **(Study #5, Montero et al. 2020. *Accepted for publication*)**, with the aim to evaluate the efficacy of different protocols for risk assessment in the detection of undiagnosed diabetes or prediabetes in a network of dental clinics (SEPA Research Network of Dental Clinics). In this study, consecutive patients attending the clinics (41 centers) were asked to fill a self-reported questionnaire (FINDRISC; (Lindstrom & Tuomilehto, 2003)) including measurements of BMI and waist circumference. In case the FINDRISC score was ≥7 (indicative of slightly elevated DM risk), participants received a point-of-care HbA1c analysed with a portable device, and those subjects with HbA1c values ≥ 5.7% were prompted to visit their physician for confirmation diagnosis. Additionally, all patients received a periodontal screening examination (EPB, from the Spanish "Examen Periodontal Básico") based on the Basic Periodontal Exam (Tugnait et al., 2004), and those subjects with a code 3, 4 or *, a complete periodontal examination.

Therefore, 1,143 individuals older than 40 years were included, of whom 97 subjects (8.5%) received a confirmatory diagnosis of diabetes (2.5%) or prediabetes (6.0%). There was a significant overrepresentation of subjects with EPB code 4 (one site with PPD≥ 6 mm) in the prediabetes and diabetes groups, confirming the assumption that periodontal status could be one of the first signs of metabolic dysregulation.

However, when the different models were assessed, the highest EPB code alone presented a low discriminating capacity (AUC=0.62), similarly to other reports using different measures of the periodontal status (A. Acharya et al., 2018; Holm et al., 2016; Lalla et al., 2013). This low predictive capacity of periodontal parameters reflects that

they should not be used as sole predictors, since it is generally considered that models with AUC<0.75 have no real clinical usefulness for the screening of undiagnosed hyperglycaemia (Fan, Upadhye, & Worster, 2006). Nevertheless, when the EPB was added to the FINDRISC questionnaire data, the performance of the FINDRISC score significantly improved (AUC=0.88). As expected, adding the point-of-care HbA1c significantly improved the screening capacity (AUC≈0.96). Although HPLC is still considered the reference method, several HbA1c point-of-care devices are available in the market, being less time-consuming (not requiring venous blood collection) and providing adequate screening capacity without the need for strict laboratory validation (Cagliero, Levina, & Nathan, 1999). In the case of the *DiabetRisk* study, we used the A1CNow$^{+®}$ device (Bayer Healthcare, Leverkusen, Germany), which has shown validation data that correlates with the HPLC reference results (Jiang et al., 2014).

Considerable variation exist in the published protocols to assess patients at risk of presenting DM in the dental setting, although most of the protocols include the application of validated risk-assessment tools combined to or not, with chairside testing of HbA1c (Harase et al., 2015; Holm et al., 2016; Lalla et al., 2013; Lalla et al., 2011). The FINDRISC questionnaire is one of the most widely used tools to evaluate the risk to develop type 2 diabetes mellitus (Lindstrom & Tuomilehto, 2003; Salinero-Fort et al., 2016; Stiglic, Fijacko, Stozer, Sheikh, & Pajnkihar, 2016), and its use has shown a good screening capacity (AUC=0.87) to discriminate between health and prediabetes/diabetes states, with an optimal cut-off established at a score ≥11 (Fan et al., 2006). The selection of FINDRISC for our protocol is, therefore, justified, since it is considered as the first step in the identification of previously unknown hyperglycaemia (Schwarz, Li, Lindstrom, & Tuomilehto, 2009), at least in Caucasian populations, which may be reasonable, considering that it is based on the most important established risk factors for this condition (e.g. age, BMI, family history). There are other risk-assessment tools, such as the Leicester Risk Assessment score, that have been validated in UK multi-ethnic populations, but that have not been previously used in Mediterranean countries (Gray et al., 2010).

The DiabetRisk Study proposes an easy protocol using minimally invasive and cost-effective methods to screen for undiagnosed hyperglycaemia in the dental setting. The potential benefits of early diagnosis and, eventually, treatment of hyperglycaemia, include the prevention of micro- and macro-vascular complications (Herman et al., 2015). Furthermore, considering the national estimates of undiagnosed DM in Spain (≈6.0%, corresponding to ≈ 2,300,000 people), the proposed protocol may help to identify approximately ≈40% of these subjects. Nonetheless, further research is needed to clarify the acceptance of this protocol for patients, dentists and physicians, as well as to estimate the potential financial savings of this approach.

The public health impact of these measures (screening of periodontitis in primary healthcare centres and screening of hyperglycaemia in the dental office) needs to be evaluated in future studies, but it seems reasonable to contend that, considering that both periodontitis and hyperglycaemia are highly prevalent and considering the periodontal-systemic inflammatory connection, oral health professionals could play a role in identifying hyperglycaemia and, conversely, physicians should be aware of periodontal diseases and their systemic implications. Synergies among healthcare providers may encourage: i) preventive programs for both periodontal diseases and hyperglycaemia; ii) early diagnosis of these conditions; and iii) treatment opportunities, with potentially better adherence by patients, leading to more desirable health and financial outcomes.

Future research considerations

It has been recognized since long time ago that DM presents a bidirectional association with periodontitis, and a large body of recent evidence from epidemiological studies supports also the association between periodontitis and metabolic syndrome. However, the glycemic control threshold over which periodontitis risk can be increased is unclear, and the same occurs for the severity and/or extent of periodontitis affecting glycemic control. Future epidemiological studies should aim to evaluate the association of different measures of abnormal glucose regulation and insulin resistance (e.g. HOMA-IR, HOMA-β, fasting plasma glucose, oral glucose tolerance) and periodontal data, including the amount and extent of attachment loss, and measures quantifying the

wound area (e.g. probing depth, bleeding on probing, etc.). Furthermore, large randomized controlled intervention trials are required to evaluate if periodontal treatment could have an impact on the prevention of diabetes complications, cardiovascular events and mortality in high risk populations, such as individuals with metabolic syndrome.

Lastly, there is increasing evidence related to the potential role of dental offices to identify subjects with undiagnosed DM, and similarly, physicians could use different screening tools to evaluate the periodontal status of their patients in light of the systemic consequences of periodontitis. In order to determine the most suitable protocols and interventions, feasibility studies including technical, economic, legal and scheduling considerations are required.

VIII. CONCLUSIONS

The present work demonstrates a significant association between certain cardiometabolic factors, such as diabetes mellitus or metabolic syndrome, and periodontitis. Furthermore, this association has additional implications from a public health perspective, since periodontal treatment leads to a reduction in surrogate measures of cardiovascular risk in these patients, highlighting the importance of early diagnosis and management of these pathologies. With that purpose in mind, different screening protocols seem to be effective in the identification of previously undiagnosed diabetes mellitus or periodontitis. Specifically:

Severe periodontitis is associated with diabetes mellitus and metabolic syndrome in the Spanish employed population, and this association is independent of body mass index and other confounders such as age, gender or toothbrushing frequency.

Periodontal therapy reduces certain measures of cardiovascular risk in patients with metabolic syndrome and severe periodontitis by significantly reducing hs-CRP levels, pro-inflammatory mediators, blood pressure and HbA1c levels.

Personalized integrative approaches by physicians and dentists for the co-management of metabolic disorders and periodontitis may be feasible. A predictive model using a combination of metabolic (HbA1c), demographic (age, gender and ethnicity) and lifestyle variables (smoking habit), may be applicable in medical care settings to screen for moderate-to-severe periodontitis.

Similarly, a protocol combining a validated questionnaire (FINDRISC) with a chairside HbA1c shows potential for the identification of undiagnosed hyperglycaemia.

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